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ALLAHABAD FARMER

The

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and

Animal Husbandry

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CONTENTS

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CONTENTS

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Original articles

Reviews

2/7/53

Vol. XXII, Part III September, 1952

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ICAR. 6. XXII. 3.
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CONTENTS

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ICAR. 6. XXII. 4.
830

CONTENTS

Vol. XXII, Part IV

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ORIGINAL ARTICLES

STUDIES ON *DAHI**

I. INTRODUCTION AND HISTORICAL REVIEW

By H. Laxminarayana and K. K. Iya, Indian Dairy Research Institute, Bangalore

(Received for publication on 8 January 1951)

DAHI (Sanskrit: *dadhi*) is an important fermentation product of milk used in this country since times immemorial as an article of diet, as a refreshing beverage and as an intermediary in the manufacture of desi butter and *ghee*. It has received great prominence in Hindu religious rites and numerous references to *dahi* can be found in the Vedas and other ancient Hindu scriptures. It also finds extensive use in the Ayurvedic system of medicine [Dutt, 1877]. As early as the second century A.D., the sages *Charka* and *Sushruta* [Kaviratna, 1922; Bhishagratna, 1907] described the nutritive value and therapeutic properties of *dahi*.

Dahi is prepared by the lactic fermentation of milk. While the coagulum obtained by souring whole milk is generally called *dahi*, the term *lassi* is used to denote either a similar product made from skim milk or the popular drink prepared by beating *dahi* into a homogenous liquid. The liquid remaining after *dahi* is churned and the butter removed, is often called *lassi*, *chass* or *mattha*, and is thus different from butter milk, which is the by-product obtained from the churning of cream in the manufacture of creamery butter.

Fermented milks

Dahi may be regarded as the Indian counterpart of Yoghurt, Kefir, and other fermented milk preparations called by a variety of local names in different countries. Most of these fermented milks are stated to have originated in the south-eastern parts of Europe, the Middle East, India and other Asiatic countries, where the tropical or sub-tropical conditions necessitated the preservation of milk from undergoing rapid spoilage by proteolytic and gas producing organisms; conversion of milk into a sour product by lactic acid fermentation offered the best means for the purpose. Accordingly, milk obtained from various species of animals, *e.g.*, cow, camel, ewe, mare, buffalo and reindeer, has been utilised in this form since the earliest ages. Various methods of providing favourable conditions for the desired type of fermentation have been in vogue, *e.g.*, (i) addition of previously soured milk or a small piece of decaying vegetable or animal matter to establish a preponderance of lactic acid organisms in the milk, (ii) holding milk at high temperatures to permit natural souring to take place as a result of the action of organism

*Investigations carried out under a Research Scheme financed by the Indian Council of Agricultural Research, New Delhi

derived from the utensil or atmosphere, or (iii) use of containers like skin bags, the unclean surfaces providing the requisite contamination of milk-souring organisms. In actual practice the fermentations are brought about by a mixture of organisms due to the primitive methods followed in the preparation of fermented milks. The numerous fermented milk preparations of different countries can be divided into two broad groups, namely, one in which acid production by lactic acid bacteria is the principal feature, with little or no alcohol formation, and the second in which alcoholic fermentation by yeasts plays an important part along with lactic acid fermentation. Some of the important fermented milks prepared in different parts of the world are indicated in Table I.

TABLE I

*Fermented milks in different parts of the world**

Name of fermented preparation	Name of country where it is used	Kind of milk used	Distinctive characteristics	Principal organisms taking part in the fermentation
(a) Predominantly lactic acid fermentation with little or no alcohol production				
Yoghurt (Bulgarian milk)	Bulgaria and Turkey	Cow's buffalo's, sheep's, or goat's milk (pasteurised or partially evaporated)	A thick curdled milk; highly acidic; contains little or no alcohol	Mainly lactic acid bacteria *L. yoghurt*, *L. bulgaricus* and *S. thermophilus*
Mazun	Armenia			
Gioddu	Sardinia			
Huslanka	E. Carpathians	Cows, buffalo's or goat's milk	....	*L. bulgaricus*, *L. yoghurt*, *S. lactis*, yeasts and Oidia
Skyr	Iceland			
Tarho	Balkans			
Mezzoradu	Sicily			
Taette	Scandinavian countries	Cow's milk (raw)	Slimy consistency	*S. lactis var. hollandicus*
Piimae	Finland	Skim milk	do.	do.
Saya	Far Eastern countries	Raw milk	Considerable amount of gas (CO_2) and proteolysis	*S. lactis* and lactobacilli
(b) Alcoholic fermentation in addition to the usual lactic acid production.				
Kefir	Caucasus, Southern Russia	Cow's, sheep's or goat's milk	Many cauliflower like grains present in a goat skin bag used for fermenting the milk; alcohol flavour	*S. lactis*, *S. kefir*, *L. casei*; *Saccharomyces kefir*; lactose fermenting torulae; contaminants such as *E. coli* *A. cloacae*, *B. subtilis*, etc. are also found
Koumiss	Siberia, Southern Russia Central Asia	Mare's milk (raw)	Preliminary acidic and later alcoholic fermentation (alcohol concentration up to 3 per cent); effervescent	*L. bulgaricus*, torulae (lactose fermenting), *B. lactie acidi*
Pauira	Lapland	do.	do.	do.

* Rogers [1916], Gasser [1931], Corminboeuf [1933], The Associates of Rogers [1935], Tanner [1944]

TABLE I—*contd.*

Fermented milks in different parts of the world—contd.

Name of fermented preparation	Name of country where it is used	Kind of milk used	Distinctive characteristics	Principal organisms taking part in the fermentation
Kuban	Southern Russia	Pasteurised milk	Slimy and alcoholic	*L. lactis var. hollandicus*, *L. bulgaricus* and yeast
Leben .	Egypt	Cow's, goat's or buffalo's milk		Lactic acid bacteria, yeasts and mycoderma
Kaelder-milk	Norway	Boiled milk	Large quantities of lactic acid (2·5 per cent) and alcohol (0·5-1·0 per cent)	Ropy milk organism
Urda	Carpathian mountains	Sheep's milk	'Sparkling whey'	Lactose fermenting yeasts and Latic acid bacteria
Skuta or (Whey champagne)	Chile	Whey	Alcoholic	Lactose fermenting yeasts and lactic acid bacteria
Busa	Turkestan		Alcoholic	do.

The first group is typified by Yoghurt (also called Yoghourt, Yaourt or Bulgarian milk) made in Bulgaria and Turkey, where it is one of the most popular fermented milk drinks. It is thick, curdled milk, which is decidedly acid and contains little or no alcohol. In this Product, the fermentation is usually brought about almost exclusively by lactic acid bacteria, which have now been identified as *Streptococcus thermophilus*, *Thermobacterium yoghurt* (Orla Jensen) and *Lactobacillus bulgaricus* [Rosell, 1933 and 1935]. These organisms by their combined activities bring about the rapid coagulation of milk. While the streptococci initiate the fermentation and are also responsible for the pleasant flavour associated with this product, the lactobacilli produce high acidity (1·0 to 2·0 per cent or even more). Yoghurt is made from either pasteurised or partially evaporated cow's milk, or in some cases from sheep's, goat's or buffalo's milk. In addition to the organisms which produce a lactic acid fermentation, there are also some peptonising bacteria present which produce a partial proteolysis of the casein [Harvey and Hill, 1948]. Occasionally a mild alcohol flavour is produced by certain lactose fermenting Saccharomyces [Rettger, 1923 ; Corminboeuf, 1932].

Tarho (Balkans), Mazun (Armenia), Gioddu (Sardinia), Mezzoradu (Sicily) Huslanka (East Carpathians) and Skyr (Iceland) are stated to be products similar to Yoghurt ; their fermentations are caused by high acid-producing rods resembling *L. bulgaricus* [Gasser, 1931 ; Corminboeuf, 1933]. Other organisms found in them include large rods resembling Yoghurt bacilli, yeasts, torulae and Oidia. It is stated that the leaves of certain plants like butter-wort are used as inoculum for

preparing Huslanka [Olsen-Sopp, 1912; Heinemann, 1911]. Taette is a sour milk used in Scandinavia and in which a slimy fermentation is caused by a variant of *S. lactis* called *S. taette* (probably *S. lactis* var. *hollandicus*). Piimae is a similar product prepared from skim milk in Finland [Gasser, 1931]. Saya is prepared in the East from unheated milk by first ripening with *S. lactis* and later with lactobacilli. A considerable amount of gas (probably CO_2) is produced and proteolysis also occurs [Gasser, 1931; The Associates of Rogers, 1935].

The second group of fermented milks includes Kefir, Leben, Koumiss, and other less known products. Of these Kefir (or Kephyr) is one of the oldest varieties of fermented milk manufactured from the milk of cows, sheep or goats. It is believed to form a considerable part of the food of mountain dwellers of the Caucasus region, who prepare it in leather bottles made from goat skin. In this product the fermentation is brought about by Kefir grains which resemble miniature cauliflowers in shape and structure and consist of casein, yeasts and bacteria. Various species of microorganisms have been isolated from Kefir, e. g., *S. lactis*, *S. kefir*, *Dispora caucasica* (*L. caucasicum Beijerinck*), *Streptobacterium casei*, *Saccharomyces kefir*, lactose fermenting torulae, *Oidium lactis*, *Bacillus subtilis* and butyric acid bacteria [Corminboeuf, 1933, Burke, 1940, Orla Jensen, 1942, Tanner, 1944]. The lactic acid bacteria produce lactic acid while the yeasts produce about 1 to 1·2 per cent alcohol. Occasionally organisms resembling *B. coli* and *B. cloacae* are also said to be present and they produce gas as well as a slimy consistency in the kefir. The fermentation is usually carried out in closed bottles so that the gas is retained and the milk becomes effervescent. Koumiss (Kumys), used extensively in Siberia, Central Asia and Southern Russia, is also prepared by a preliminary acidic and subsequent alcoholic fermentation of unpasteurised mare's milk. It has a higher concentration of alcohol (about 3 per cent) than Kefir and is a highly effervescent drink. The fermentation is due to *L. bulgaricus* principally while lactose fermenting torulae and *Bacterium lactis acidi* (Leichmann) play a secondary part [Voitkevich, 1934; Tanner, 1944]. Pauira, a product similar to Koumiss is used in Lapland [Gasser, 1931]. Kuban, a product used in Southern Russia is made from pasteurised milk by a combined lactic and alcoholic fermentation. The microflora include a lactic streptococcus resembling *S. lactis* var. *hollandicus*, a lactic acid rod of the *L. bulgaricus* type and three types of yeasts [Bogdanoff, 1934]. Leben is another fermented milk prepared by Egyptians from the milk of cows, goats or buffaloes and the fermentation is brought about by the joint action of lactic acid bacteria and yeasts [Khoury and Rist, 1902]. Kaelder-milk (cellar milk) is a Norwegian product prepared by inoculating boiled milk with a special variety of ropy milk and keeping it cold. Large quantities of lactic acid (2·5 per cent) and some alcohol (0·5 per cent) are formed which enable the product to keep well [Rettger, 1923]. It is often used as a substitute for fresh milk by Norwegian peasants. Urda is a strongly alcoholic beverage prepared in the Carpathians from sheep's milk, and sometimes known as Sparkling whey. Whey champagne or Skuta is a similar product prepared in Chile by the fermentation of whey through the agency of lactose fermenting yeasts and some species of lactic acid bacteria [Rettger, 1923; Harvey and Hill, 1948]. Another variety of fermented milk containing up to 0·78 per cent of lactic acid and 7·1 per cent of alcohol is used in Turkestan under the name Busa [Chekan, 1922].

In recent years special fermented milks under the name 'cultured milks' have become very popular in Europe and America. The most important of these are 'Bulgarian butter milk' and 'Acidophilus milk'. Bulgarian butter milk is prepared by souring skimmed or whole milk with a pure culture of *Lactobacillus bulgaricus* and the product is viscous and highly sour [Burke, 1938]. In the preparation of Acidophilus milk pure cultures of *Lactobacillus acidophilus* are used and the product is not as sour as Bulgarian butter milk [Knaysi, 1932]. It is greatly valued for its reputed therapeutic properties. Pure cultures of *Streptococcus paracitrovorous* are sometimes used for the development of desirable flavour in cultured milks. In order to improve the taste and nutritive value of the products various fruit juices and special flavouring substances are sometimes incorporated. Several modifications, involving the use of skimmed milk, whole milk, cream, condensed and dried milks or whey, and the manufacture of refrigerated or iced butter milk and dried butter milks constitute some of the latest developments in this field of dairy industry [Burke, 1938; Cronshaw, 1947].

It is evident that in the various fermented milk preparations lactic acid bacteria are the principal agents taking part in the fermentative processes. The fermentation of lactose into lactic acid is the primary chemical change sometimes accompanied by the production of volatile acids and gas or a mild alcoholic fermentation brought about by yeasts. There may be a slight increase in the soluble nitrogen content due to the action of microorganisms on proteins. Van Slyke [1928] and recently Khambatta and Dastur [1950] investigated the various changes in the constituents of milk occurring during the process of souring. The lactic acid bacteria occurring in fermented milks can be divided into three main groups according to the latest taxonomical classification of Bergey [Breed and others, 1948]. The first group includes the common milk souring organism, *Streptococcus lactis*; the high temperature milk souring streptococcus, *L. thermophilus*; and *S. cremoris*, used for the ripening of cream. They produce mainly lactic acid by the fermentation of lactose, the final acidity reached being one per cent calculated as lactic acid. The second group includes the high acid producing lactobacilli, *L. caucasicum, L. bulgaricus, L. acidophilus. L. casei and L. plantarum.* The first two species are most commonly associated with the sour milks, while *L. acidophilus* is primarily an intestinal organism, whose presence in fermented milks is considered of therapeutic significance. All these lactobacilli ferment lactose mainly to lactic acid, usually up to 2·5 per cent or rarely 3·5 per cent titratable acidity, and on account of their higher acid tolerance they always predominate over streptococci in course of time. The hetero-fermentative lactobacilli (Betabacteria), which grow slowly and produce acetic acid, propionic acid and other volatile compounds as well as carbon dioxide and hydrogen in addition to lactic acid, come under this group. The third group includes the so-called 'aroma bacteria'—*S. citrovorous* and *S. paracitrovorous*, which are slow acid producers and can only grow in association with other lactic streptococci. They produce lactic acid together with volatile acids and gas but their main importance consists in their ability to form acetyl methyl carbinol (acetoin) and its oxidation product diacetyl by the fermentation of citric acid or citrates in milk. The formation of diacetyl is considered to be responsible for the characteristic aroma associated with many fermented milks. Next in importance to lactic acid bacteria are the yeasts (Saccharomycetes and Torulae, as well as

some Mycoderma species) and fungi which are frequently present in sour milk products as the acidic environment is favourable for their growth. The yeasts produce smaller or larger amounts of alcohol together with carbon dioxide by the fermentation of lactose or its break-down products, glucose and galactose. Various types of fungi, of which species of Oidium are perhaps most frequently encountered, may cause secondary fermentations associated with the decomposition of casein and fat. Members of the genus Bacillus (aerobic spore-formers), butyric acid bacteria and coliform organisms are frequently responsible for the development of off-flavours, gassiness and other defects in the product and they are to be regarded as undesirable contaminants in fermented milk preparations.

Some data available regarding the composition of a few fermented milks are presented in Table II.

TABLE II

Chemical composition of some fermented milks

—	Water	Total solids	Fat	Protein	Lactose	Ash	Lactic acid	Alcohol	Carbon dioxide
	(Figures are in per cent)								
Yoghurt	73·69	26·31	7·20	..	9·40	1·38	0·80	0·20	..
Kefir	88·2 to 89·0	..	2·8 to 3·3	..	1·7 to 2·9	..	0·60 to 0·90	0·6 to 1·1	..
Leben[3]	86·23	13·77	3·38	4·74	3·15	0·69	1·69	..	..
Tarho[4]	88·25	11·75	1·40	..	4·50	0·85	2·00	0·37	..
Taette[1] (6 weeks old)	88·04	..	3·36	2·84	3·67	0·65	1·44	0·37	0·60
Koumiss[1] (separated cow's milk).	83·93	..	0·85	2·02	3·11	0·44	0·80	2·65	1·03
Koumiss[1] (mare's milk)	91·53	..	1·27	1·91	1·25	0·29	1·01	1·05	0·88

[1]Gasser [1931] [2]Voitkevich [1934] [3]Aigues [1928] [4]Gratz [1930]

Therapeutic and nutritional significance of fermented milks

The popularity of fermented milks amongst many peoples is due to their reputed therapeutic properties as well as nutritional significance, in addition to their use as refreshing beverages. They also offer a means of preserving milk in a suitable form for human consumption over longer periods than is possible with sweet milk. The importance of *dahi* and *lassi* for the maintenance of health and in the treatment of gastrointestinal disorders has been long recognised in India. Both Sushruta and

Charaka, the great exponents of Ayurvedic system of medicine in the 2nd century B.C., have referred to the value of curds in promoting appetite, in increasing the vitality, in curing dyspepsia, diarrohea, nasal catarrh, dysentery, intermittent fever and other diseases and in maintaining the balance of the three bodily humors *viz.*, Vayu, Pittam and Kapham [Bhishagratna, 1907; Kaviratna, 1922]. They, however, warn that improperly prepared curds may be harmful to the system. *Takra* (butter milk prepared by adding one fourth part of water to whole milk curds) has been described as having cooling, appetizing and tonic properties and as useful remedies in cases of diarrhoea, dyspepsia, urinary diseases, etc. The above authors also consider that the habitual use of butter milk is conducive to better health and increases resistance to diseases, although it is not recommended for the weak or tuberculous patients nor for those suffering from fever and nervous debility.

Metschnikoff [1901] and Douglas [1911] were perhaps the earliest European workers to indicate the value of fermented milks in intestinal disorders. Metschnikoff put forward the theory that the decay of the human body was due to the absorption of toxins resulting from microbial protein decomposition in the intestines and that such putrefactive processes could be controlled by the use of butter milk, especially Bulgarian milk, resulting in imporved health and longevity. The Bulgarian bacillus (*L. bulgaricus*)—the souring organism in Bulgarian milk—could be transplanted and developed in the intestinal tract by daily ingestion of fermented milk. The high acidity produced by these organisms was responsible for inhibiting the growth of putrefactive organisms in the intestine. It was subsequently established [Rettger, 1915; Kopeloff, 1925] that *L. bulgaricus* could not be propagated in the intestinal tract but that the Acidophilus organism (*L. acidophilus*), which was a normal inhabitant of intestinal tract, could be induced to grow there by creating favourable conditions for it. Myers [1931] demonstrated that the most important consideration for successful implantation was the use of a proper strain of *L. acidophilus* adapted for growth in the intestine. Kopeloff [1923] has stated that patients taking Acidophilus milk containing viable cultures of the organism were relieved from constipation. Apart from the role of the added lactic acid organisms, the acid of soured milk and the lactose content have also been considered as important factors contributing to the therapeutic value of fermented milks [Rettger, 1917; the Associates of Rogers, 1935]. The value of fermented milk preparations in bringing relief to persons suffering from specific gastro-intestinal disorders, such as chronic constipation, diarrhoea, etc., has been experimentally verified by a number of workers. Kopeloff [1925] and Lonergan and Beerman [1925] fed Acidophilus milk to epileptic patients and were able to control the number of convulsions, although the usefulness of this treatment in curing epilepsy was not confirmed by Lynch [1928]. Smith [1924] was able to transform 80 to 90 per cent of the intestinal flora of typhoid and paratyphoid carriers to *L. acidophilus* by feeding Acidophilus milk. The inhibitory influence of fermented milks on pathogenic bacteria such as *B. coli*, *S. dysenteriae*, *E. typhosus*, Salmonella species, *B paratyphi*, *B. abortus*, etc., has been reported by various workers [Fisher, 1920; Kazaryan, 1936; Sircana, 1937; Nicholls and associates, 1939; Kazberynk, 1940; Bhat and Reporter, 1949]. Kliewe and Schuppener [1937] observed that M. *tuberculosis var. hominis* survived for 7 days and the bovine

variety for 20 days in sour milk. Mattick [1946] has made some interesting observations regarding the destruction of tubercle bacilli in sour milk. While further investigation in necessary for determining the bactericidal action of fermented milk on specific pathogens, the concensus of opinion is in favour of regarding sour milk as a poor culture medium for the growth of pathogens and as a much safer food product to handle than sweet milk even where sanitary methods are not practised.

The food value of fermented milk is considered to be more or less similar to that of milk from which it is prepared. The sugar content is slightly reduced due to its fermentation into acid, alcohol or gas, while the casein is precipitated in a readily digestible condition. The increased digestibility of fermented milk is attributed to the finely divided state in which casein is available [Orla Jensen and Spur, 1924]. These milks are, therefore, frequently used in cases of gastric irritation when it is difficult to find foods which can be retained by the stomach. The lactic acid in these products is said to be completely metabolized to carbon dioxide and water in man and is not excreted in the urine nor does it have any effect on the acid—base balance in the system (White House Conference on Child Health and Protection, 1932). Davies [1940] has referred to two other possible advantages of drinking fermented milks, namely, (*a*) the increased availability of calcium and phosphorus from calcium precipitated in the lower intestines due to the acid condition brought about by the lactic acid organisms, and (*b*) the increased efficiency of the body to cope with comparatively large quantities of lactic acid entering the system. It has also been shown [Wegner and associates, 1940 ; Welch and Wright, 1943] that some of the organisms occurring in fermented milks are able to synthesize certain vitamins and if such organisms are also present in the intestines they may play a significant role in the synthesis of some of the vitamins required by the host. Gasser [1931] has reported that Saya (a fermented milk of the East recently introduced on the Continent) is rich in vitamins A, B, C, and D and is superior to Kefir, Yoghurt or fresh milk. Chitre and Patwardhan [1945] found the riboflavin content of curd to be greater than that of the milk from which it was made. There was no appreciable change in thiamine, but nicotinic acid showed a slight decrease. Recent work on these aspects has been reviewed by Kon and Henry [1949].

Composition of dahi

Dahi is a thick, sour, coagulated milk product bearing a close resemblance to Yoghurt in many respects. It is prepared either from cow's or buffalo's milk, while *lassi* is made from skim milk. Generally, cow's or buffalo's milk is boiled or simmered for a long time to concentrate it, allowed to cool to body temperature and then transferred to earthenware pots or pans. A small amount of the previous day's curd is added as a starter and the inoculated milk is incubated at room temperature or at a temperature of 30° to 37°C., according to convenience. The curd is usually ready in 16 to 20 hours. Occasionally raw milk may be employed or heated milk may be allowed to sour naturally without the addition of any starter.

Davies [1940] has discussed some of the factors involved in the manufacture of *dahi* and *lassi*. The following ranges of composition for normal samples of *dahi* and *lassi* have been reported by him in Table III.

TABLE III

Chemical composition of dahi

—	*dahi*	*Lassi*, skimmed milk	*Lassi* butter milk
	(per cent)	(per cent)	(per cent)
Water	85—88	90—91	90—91
Fat	5—8	0·05—0·10	0·1—1·0
Protein	3·2—3·4	3·3—3·5	3·3—3·5
Lactose	4·6—5·2	4·7—5·3	4·7—5·3
Ash	0·79—0·75	0·7—0·75	0·70—·75
Calcium	0·12—0·14	0·12—0·14	0·12—0·14
Phosphorus	0·09—0·11	0·09—0·11	0·09—0·11
Lactic acid	0·5—1·1	0·5—0·1	0·5—0·1

The composition of butter milk has also been studied by Ranganathan and associates [1937], Ranganathan and Narasimhamurthy [1938] and Chitre and Patwardhan [1945]. These workers have also compared the amount of certain vitamins contained in fresh milk with those in curdled milk made from it.

Microbiology of dahi

As in Yoghurt, lactic acid fermentation is the basis underlying the production of *dahi* but the information available regarding the microbiology of *dahi* and the nature of fermentations taking place is very meagre. Chatterjee [1910] was perhaps the first worker to isolate a lactic acid organism from *dahi*, which closely resembled the high acid producing lactobacillus included in the *L. caucasicus* group and which he named *Streptothrix dadhi*. 'Ram Ayyar [1928, a] described a similar organism predominating in *dahi* prepared during hot weather. According to him, *dahi* prepared in winter was less sour and the curdling organism was found to be a short rod occurring in pairs and similar in appearance to starter streptococci. Yeasts of the torula type were always found associated with both types of lactic acid organisms and were considered to be responsible for imparting a peculiar flavour to the product and for peptonising the curd slowly. They did not appear to form any alcohol." In a subsequent communication [Ram Ayyar, 1928 b] this worker described a lactose-fermenting yeast occurring in some *dahi* samples, which was able to produce ethyl alcohol up to two per cent. Joshi and Ram Ayyar [1936] isolated a streptococcus from *dahi* which was capable of curdling milk quickly and also producing good aroma (combining the characteristics of *S. lactis* and *S. paracitrovorous*) and they suggested the name *S. lactis aromaticus* for it. A similar organism was isolated from *dahi* by Karnad [1939] which was called *S. diacetyl aromaticus* in view of its ability to produce up to one per cent of lactic acid together with high amounts of

acetoin and diacetyl. Pasricha and associates [1938] examined bacteriologically some 24 samples of bazaar *dahi* while Madhok and Kapoor [1942] carried out some experiments on the preparation of *dahi* using pure cultures of lactic acid bacteria singly and in combination.

From the foregoing review it is evident that *dahi* constitutes a highly nutritious food for the people, apart from its usefulness as a refreshing drink or as a possible therapeutic agent. It provides a satisfactory means of utilising surplus milk economically and of preserving the food value of milk over considerable periods under the tropical conditions prevailing in this country. *Dahi* is prepared in practically every cultivator's house on a cottage industry basis ; by churning it and selling the butter or *ghee* made from it the cultivator earns ready cash while the by-product, *lassi*, forms a palatable and nutritive drink or article of diet for the use of the family. It is estimated that over 2,069·4 million gallons of milk or 52·4 per cent of the total net annual production of milk in the country is converted into *dahi*, most of which is utilised for manufacture of *desi* butter and *ghee* [Agricultural Marketing Adviser, 1949]. Large quantities of *lassi* (butter milk) are produced annually as a by-product. The quantity of milk used for making *dahi* for direct consumption is estimated at 359·1 million gallons or 9·1 per cent of the total production of milk per year. The manufacture of *dahi* is, therefore, of immense economic importance both to the individual producer as well as to the dairy industry as a whole. There is also considerable scope for the economic utilisation of large quantity of separated milk, formed as a by-product in the separation of cream for butter manufacture, by converting it into *dahi*, thus adding to the food resources of the nation.

The existing conditions of *dahi* manufacture in India are, however, far from satisfactory and the quality of the product available in the market is inferior as well as highly variable, except for a few isolated cases. Obviously, this important branch of the dairy industry has not been properly exploited either for meeting the nutritional needs of the people or improving the economic condition of the producers. An additional factor to be considered in this connection is the ultimate effect of using *dahi* of poor quality in *ghee* manufacture since the flavour and keeping quality of ghee are said to be influenced by the quality of *desi* butter which in turn is governed by the quality of *dahi* [Rangappa and Banerjee, 1946].

Scope of present study

The above considerations are sufficient to indicate the importance of *dahi* and *lassi* manufacture in India and the need for effecting improvements in the existing methods of production and marketing so as to ensure a uniformly high standard in the quality of *dahi*. The formulation of any step for improving the production of *dahi* should, however, be based on a proper appreciation of the factors which go to determine the quality of *dahi*, such as the types of organisms associated with *dahi* produced in different parts of the country and their characteristics, influence of climatic and seasonal variations, quality of milk used and method of manufacture, about which there is very little information available. The present study was undertaken with a view to (a) collect systematic information on the above aspects, (b) prepare pure culture starters containing desirable organisms for the manufacture of *dahi*, (c) build up technical data for guidance in the preservation

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and proper utilisation of the starters for obtaining good quality *dahi* and (d) standardize the technique of *dahi* manufacture. For convenience, the results of the investigation are presented in four parts (parts II, III, IV and V) of the series. The first one deals with the qualitative survey of the available types of market *dahi*; the second one pertains to the isolation and taxonomical classification of lactic acid bacteria associated with *dahi*; the third one deals with the detailed biochemical studies on the pure cultures of *dahi* organisms; and the last one describes the preparation and utilisation of starters for different purposes and standarization of technique of *dahi* manufactur e.

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STUDIES ON *DAHI**

II. GENERAL SURVEY OF THE QUALITY OF MARKET *DAHI*

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The object of the survey was to obtain information on the composition, flavour, texture and microbial content of *dahi* produced in different parts of the country. For this purpose, several zones, broadly representing the different climatic and other natural conditions in the country and also including areas famous for quality of *dahi*, were marked out on the basis of information supplied by provincial authorities and private sources. The research workers visited important urban centres as well as rural areas in the different zones and collected representative samples of *dahi* and *lassi* from dairies, *halwais*, street vendors, private houses and other sources.

The programme for the collection of samples from different zones was so arranged as to cover all the seasons of the year. Altogether about 1,000 samples of *dahi* and *lassi* were secured for the investigation from the following centres (Table I).

Table I

Geographical distribution of dahi *samples collected for the investigation*

Zone	Centres	Number of samples	
North Indian plains	Patna, Muzaffarpur, Darbhanga (Tirhut Valley), Gorakhpore	60	
	Allahabad, Banaras, Lucknow, Kanpur, Mathura, Agra	159	
	Delhi, Meerut, Aligarh, Khurja	104	
	Karnal, Ambala, Ferozepur, Hissar	72	
	Lahore, Lyallpur	50	
	Nagpur, Bhusaval	23	
	Hyderabad (Sind)	19	
	Ahmedabad, Baroda	26	529
	Cuttack, Vijayavada	16	
Sea Coast areas	Bombay	116	
	Calcutta	74	
	Karachi	42	
	Madras	40	272
Deccan Plateau (South India)	Poona	20	
	Hyderabad (Dn.)	24	
	Bangalore, Mysore, Mandya, Hassan	73	
	Coimbatore	17	134
Areas of high altitude	Dehra Dun*, Hardwar, Rishikesh	45	
	Mahabaleswar	15	60
			995

* Investigations carried out under a research scheme financed by the Indian Council of Agricultural Research, New Delhi.

Methods

Collection of samples

All samples were collected in sterile, glass stoppered 8 oz. bottles, after mixing well the lots of *dahi* from which they were drawn. After their preliminary examination on the spot, such of the samples believed to be representative of the locality were securely packed in specially constructed insulated wooden boxes and despatched to Bangalore for further analysis.

Since the samples of *dahi* were to be utilised for chemical analysis as well as for the isolation of lactic acid bacteria, the transportation to Bangalore of the samples in their original condition presented some difficulties, particularly in the case of more distant centres. The use of insulated ice boxes for this purpose was not found to be practicable. The samples intended for chemical analysis were, therefore, preserved by adding 0·5 ml. of a 40 per cent solution of formaldehyde to each half pound sample of *dahi*. Preliminary trials showed that with such an addition, the acidity, fat, protein and ash values of the samples were not appreciably altered on keeping the sample for 5 to 6 days at room temperature (25±30°C.). Lactose determinations were not made on these samples. For bacteriological work, in addition to using the original unpreserved sample, a small amount of the sample was inoculated into dextrose agar stabs. In the case of such samples of *dahi* as were believed to require careful bacteriological examination, duplicate stabs were made of the original samples to guard against any possible mishaps.

Preliminary examination of the samples

A preliminary examination of *dahi* samples was made at the sources of collection in regard to (i) their physical appearance, texture and flavour and (ii) microscopic appearance of smears, which were fixed, defatted with xylol and stained with borax methylene blue for estimating the types and relative numbers of organisms present in the samples.

Wherever laboratory facilities permitted* the titratable acidity and total bacterial counts of the samples were also determined according to the following procedures.

(*a*) *Titratable acidity.* Ten grams of *dahi* was emulsified in 40 ml. of distilled water and titrated against 0·1 N/NaOH using phenolphthalein as indicator. The results were expressed as per cent lactic acid.

(*b*) *Total bacterial count.* The total bacterial counts were determined by using suitable modifications of standard methods (British Ministry of Health, 1939).

*Laboratory facilities were provided at the Veterinary Colleges in Madras, Calcutta, Patna, Lahore and Bombay; Agricultural College, Coimbatore; Provincial Hygiene Institute, Lucknow; Agricultural Institute, Allahabad; Cattle Farm, Hissar; Indian Agricultural Research Institute, New Delhi; Medical College, Mysore and Indian Dairy Research Institute, Bangalore.

After the samples were vigorously shaken to break the clumps of curd, small quantities (approximately 1 gm.) were weighed into sterile flasks, diluted suitably in saline and then plated in duplicate using milk agar. The plates were incubated at 37°C. for 72 hours and the colonies counted. The arithmetic average of duplicate plate counts was employed for calculation of total bacterial counts per gram of *dahi*.

Chemical analysis of the samples

Upon arrival at Bangalore, the samples of *dahi* were analysed for fat, ash, and protein by employing modifications of the procedures described in A.O.A.C. [1940]. Samples which were received in an unsatisfactory condition due to clumping of fat, considerable whey separation, etc., as a result of the heavy joltings during transit, were not used for analysis. Lactose was determined only in *dahi* samples collected from places near about Bangalore and which had not been preserved with formalin.

Fat. Fifty grams of *dahi* were mixed well with 2 ml. of strong ammonia and made up to 100 ml. with distilled water. Eleven ml. of the solution were taken and fat determined by the Gerber method. The fat percentage of *dahi* was calculated by multiplying the observed reading by two.

Ash. A 10 gm. portion of the sample was used for determination of the ash by the usual procedure [A.O.A.C. 1940].

Protein. Total nitrogen was estimated by the usual Kjeldahl method, using a 5 gm. sample of *dahi*, and the percentage of protein was calculated employing 6·3 as the conversion factor.

Lactose. Lactose was determined (according to Münsen Walker's gravimetric method) only for a few samples collected locally and from places near about Bangalore. Due to the uncertainty of the age of the samples, and the long interval in transit, extremely low values were obtained for the samples particularly in the case of those obtained from distant places. Since such values of lactose were not considered to be of practical significance, the determination of this constituent for subsequent samples was discontinued.

Methods of manufacture and other particulars of the samples

Information regarding the history of the samples with special reference to the type of milk used, process of manufacture, etc., was recorded wherever possible. It was generally reported by those from whom the sample was collected that some 12 to 24 hours had elapsed since the addition of the starter to the milk. In the case of a number of samples, particularly those obtained from south India, the high degree of souring as well as yeasty smell shown by them indicated that the information regarding age of the samples was not accurate and that they were mucholder.

There were considerable variations in the methods of manufacture, conditions of storage and in the methods of marketing of *dahi*. Either cow's milk, buffalo's milk, skimmed milk or mixtures of these were employed. Buffalo's milk was commonly used where *dahi* was mainly utilised for *ghee* manufacture. In Bengal, Bihar and eastern parts of Uttar Pradesh, cow's milk was almost always used for *dahi* making. The milk was usually brought to a boil and allowed to cool to 37°

4 A

to 38°C. (body warmth), before the addition of starter. In some cases, milk was boiled and kept simmering at a temperature of 80° to 90°C. on a low flame for 2 to 4 hours in order to concentrate the milk. This was, for example, a common practice in Tirhut Valley and in many other places in North India. In some parts of south India, however, raw milk or a mixture of morning milk and previous evening's balance of production, were used. After warming the milk, it was generally stored in unglazed earthen-ware pots or shallow earthen-ware pans. Stoneware jars, tinned brass utensils and aluminium vessels were also in use at some places. A small amount of starter was then mixed with a small quantity of milk and added to the bulk, the proportion of the starter varying from 1 to 10 per cent of the quantity of milk. The *halwais* in Calcutta reported that they added about 1 to 2 per cent of starter to milk. After addition of the starter, the milk was stirred well and set aside for curdling, with or without a cover, usually near the fire place. In order to retain the warmth of the vessel in which the milk was held, it was sometimes wrapped in cloth or with hay or the vessel was placed inside a packing of straw or hay-box. The curd was usually ready for sale in 10 to 16 hours and some *halwais* in Calcutta claimed to have prepared *dahi* in about 4 hours after the addition of the starter. A number of factors, such as the vigour of the starter employed, concentration of milk prior to addition of starter, and the temperature of incubation may have helped in accelerating the curdling.

In some centres, such as Calcutta, a few shops sold sweet *dahi* containing sugar, although generally sugar was not added to *dahi* but was left for the consumers to add according to taste. For making sweetened *dahi* the method employed usually was to add 8 to 10 per cent of cane sugar either at the time of boiling the milk or soon after.

In addition to the use of skimmed milk in making *dahi* and passing the product off as that prepared from whole milk, the practice of diluting the milk with water to different degrees before boiling and before adding the starter, was also widely prevalent. Addition of various starchy materials for improving the body of *dahi* and the addition of flavouring substances to enhance its sale value was also not uncommon. It was not possible to obtain greater details regarding these aspects from the *halwais*. In South India, where the consistency of the *dahi* or *lassi* sold was highly watery, it was evident that the villager was either adding water to *dahi* at some stage before its sale or was removing the richer top layer of the milk for making butter.

Generally, the starter employed was a portion of the previous day's batch of *dahi*. In centres, where *dahi* production was carried out in an organized manner, a portion of the curd was removed when fresh, mixed with a small quantity of boiled milk, and kept over till it was used for the preparation of the next day's batch of *dahi*. The practice of adding the whole batch of surplus curds to milk was encountered in rare instances. The system of propagating starter cultures, independently of the *dahi*, was not found to be in vogue anywhere. In many places raw milk itself was allowed to sour naturally without the addition of any starter. The starter organisms in such cases may be expected to have been derived either from the atmosphere or from the utensils or from milk itself.

The sale of *dahi* in containers exposed to atmosphere was a very common practice of the *halwais* in North India. Such shops were situated in crowded streets and *dahi* was kept in open shallow vessels exposed to dust, flies and to splashes of rain-water in monsoon. There was very little attention paid to the cleanliness of the hands or containers used at the time of selling either by the vendor or by the buyer. In the south, it was more common to find the villagers bringing curd into urban areas and selling it from door to door. Here too, there was a complete absence of hygienic practices in the handling of *dahi*.

Results

The results of the preliminary examination of *dahi* samples in regard to texture, flavour and microscopic appearance are given in Table II. The percentage distribution of the samples according to the characteristics shown by them is given in the table. The results of plate counts and acidity, which were also obtained during the preliminary examination of *dahi* at the sampling centres, are presented along with the results of chemical analysis in Table III.

It was observed that a majority of the samples of *dahi* collected in South India were thin and watery in consistency with lumps of curd floating occasionally in them, irrespective of the time of year. They had also a pronounced acid smell as well as a sour taste, some of the samples being extremely sour. Very few samples collected in summer and not more than 10 to 30 per cent of those collected in winter (particularly those obtained near Madras or Mysore) possessed a good texture as well as the typical *dahi* flavour. In 15 to 25 per cent of the samples, peptonisation of the curd, gas formation and disagreeable flavours were noticed while estery or alcoholic flavours were noticed in a few instances.

The samples of *dahi* collected from Poona, Bhusaval and Nagpur also contained a high proportion of sour *dahi* but most of them showed fairly good texture. On the other hand, the samples from Gujarat were generally very poor both in regard to body and in flavour, a few samples from Baroda being the only exceptions.

About 50 per cent of the samples collected in Bombay showed good physical texture, sweet or mildly sour taste, and pleasant aroma. Some 30 per cent of them were sour and the rest were associated with various defects. There was not much difference between the winter and summer *dahi*, except that the latter included a greater number of samples (mostly collected during monsoon) having estery or alcoholic flavours as well as showing peptonisation and gas formation.

As regards the Calcutta samples, nearly 60 per cent of those collected in winter had very good texture together with a pleasant flavour, the rest were either watery or associated with off-flavours. Most of the summer samples had a poor texture. Some 30 per cent showed the characteristic flavour of *dahi*, 50 per cent were sour and about 10 per cent were associated with estery or alcoholic flavours. A large number of the Calcutta samples were also sweet to taste and in some cases this was reported to be due to the sugar added to them.

Almost all samples collected from areas in Tirhut Valley, Uttar Pradesh, Delhi, Punjab, and Sind, irrespective of season, were characterized by good curd formation

TABLE II

Distribution of dahi *samples (expressed as per cent) collected from different areas according to their texture, flavour and microscopic appearance*

Area (Ref: Table-I)	Texture				Aroma				Taste				appearance			
	Solid, uniform, smooth.	Viscous with lumps of curd.	Watery with curd particles.	Peptonized or gassy.	Typical, aromatic.	Acidic.	Estery of alcoholic.	Flat or unpleasant.	Sweet.	Mildly sour.	Very sour	Bitter, oily or disagreeable	Streptococci dominant	Lactobacilli dominant	Cocci and rods equal	Yeasts numerous
Winter																
Patna	63	24	2	11	58	24	2	16	53	31	9	7	46	18	36	30
Allahabad	75	16	4	5	65	25	7	3	68	24	7	1	60	7	26	19
Delhi	80	18	0	2	81	13	1	5	77	20	2	1	75	0	25	26
Karnal	78	18	4	0	67	15	7	11	76	17	3	4	72	8	20	24
Lahore	82	10	0	8	86	12	0	2	74	26	0	0	84	6	10	26
Hyderabad (Sind)	66	22	0	12	75	24	0	1	72	18	10	0	60	20	20	25
Nagpur	55	22	0	23	22	45	8	25	18	45	20	17	18	28	54	46
Ahmedabad	20	26	22	32	22	40	0	38	23	32	23	22	8	40	52	50
Poona	40	25	15	20	35	30	0	35	30	40	15	15	0	0	100	80
Hyderabad (Deccan)	0	33	48	19	4	80	4	2	9	18	56	17	0	68	32	56
Bangalore	5	13	71	11	13	72	5	16	9	23	55	11	3	69	28	82
Coimbatore	6	18	48	28	18	84	0	0	12	30	58	0	0	66	34	90
Madras	20	20	30	20	35	25	5	35	30	35	55	20	0	90	10	87
Bombay	43	23	18	16	49	28	10	13	46	28	16	10	37	28	35	82
Karachi	86	9	3	2	80	16	2	2	88	9	3	0	84	3	13	15
Calcutta	61	18	13	8	57	22	0	21	59	22	11	8	74	11	15	18
Dehra Dun	90	10	0	0	86	3	0	6	85	15	0	0	75	8	17	10
Mahabaleswar	52	13	25	10	40	26	20	14	40	36	20	4	20	40	40	27
Summer																
Patna	60	27	6	13	47	40	0	13	40	33	20	7	20	40	40	40
Allahabad	72	16	1	11	70	23	0	7	65	24	10	1	42	34	34	50
Delhi	91	8	0	1	75	18	0	4	72	18	10	0	46	26	27	48
Cuttack	0	60	20	20	0	100	0	0	0	80	20	0	0	100	0	100
Poona	40	30	20	10	40	50	10	0	0	40	30	20	0	60	40	30
Bangalore	20	10	40	30	0	50	0	20	0	0	30	20	0	90	90	50
Madras	10	10	75	25	20	55	10	15	5	60	30	15	0	90	10	70
Bombay	54	20	16	10	24	25	8	15	42	25	16	17	17	68	15	35
Calcutta	13	33	12	17	23	42	11	14	67	21	7	5	21	23	36	75

TABLE III

Bacteriological and chemical analysis of dahi *samples (figures in the table represent the ranges of variation in the values)*

area (Ref : Table-I)		Number of samples	Total bacterial count per gm. of *dahi* (millions)	Titratable acidity (per cent lactic acid)	Fat (per cent)	Ash (per cent)	Protein (per cent)	Lactose (per cent)
Bangalore	(W)	38	88—672	0·30—1·39	0·14—2·20	0·22—0·76	1·29—4·43	0·22—3·57
	(S)	25	100—500	0·49—0·94	0·30—6·90	0·26—0·69	1·39—3·26	0·09—1·09
Coimbatore	(W)	17	226—456	0·70—1·78	0·80—4·80	0·44—0·72	2·28—4·54	0·10—1·48
Hyderabad (Dn.)	(S)	12	*	*	*	0·38—0·50	3·00—3·60	*
Poona	(W)	9	*	*	0·84—4·0	0·48—0·51	3·46—3·91	*
Madras	(W)	20	35—438	0·60—1·70	0·10—1·03	0·42—0·96	2·18—3·20	0·07—0·59
	(S)	20	*	1·32—1·95	0·20—0·30	0·34—0·57	2·28—3·27	*
Calcutta	(W)	10	*	*	2·40—4·80	0·58—0·85	4·03—4·97	*
	(S)	47	*	0·58—1·44	2·80—7·40	0·44—0·85	2·60—4·10	*
Bombay	(W)	39	*	1·64—2·15	2·90—9·10	0·47—0·70	2·94—4·10	*
	(S)	30	18—730	1·12—1·72	2·10—8·20	0·47—0·69	3·54—4·77	*
Karachi	(W)	22	216—406	1·09—1·31	*	*	3·96—5·15	
Patna	(W)	17	*	*	1·20—4·40	*	*	*
Allahabad	(S)	29	*	1·15—1·42	1·40—8·40	0·49—0·85	3·74—5·17	*
Delhi	(W)	36	13—22	1·12—1·45	5·10—9·00	0·46—0·68	3·58—5·30	*
Karnal	(W)	17	*	*	6·40—8·60	0·48—0·70	3·87—5·16	*
Lahore	(W)	22	319—560	1·38—2·09	3·30—5·50	0·37—0·66	3·63—4·80	*
Cuttack	(S)	5	99—232	1·05—1·18	2·80—4·60	0·50—0·78	3·15—4·83	*

* Not determined (W) Winter samples (S) Summer samples

and uniform texture with a sweet to mildly sour taste and pleasant aroma. Only a small percentage of the samples showed any peptonisation, gas formation or other defect even during summer. Some of the best samples for the whole series were obtained from Darbhanga and Brindavan (Mathura). Many of them were markedly sweet although there was no evidence of any extraneous sugar having been added to them.

There is thus a considerable variation in the quality of market *dahi* obtained in different centres and in some centres in different seasons of the year. The north Indian samples as a class possessed a better texture and flavour than their south Indian counterparts, which were generally poor in consistency and were highly acidic in flavour. While this difference may be mainly attributed to the quality of raw material used as well as the methods of manufacture described earlier, the types of microflora occurring in the samples should also be considered. As a matter of fact, the microorganisms would be responsible for the summer samples being more acidic than the winter ones in many places and for a large number of samples collected during the monsoon season, particularly in Bombay and in Calcutta, showing estery or alcoholic flavours together with peptonisation, gas formation, and other defects.

Most of the south Indian samples showed a preponderance of lactobacilli and yeast cells over the cocci. Although streptococci were present in some of them they must have been over-grown by the more acid tolerant and high acid producing lactobacilli due to the nature of the starter, effect of age and temperature. This may account for the high degree of souring in these samples. A few samples which showed a sweeter flavour contained streptococci in greater abundance. On the other hand, streptococci were predominant in the north Indian samples, with lactobacilli and yeasts occupying a secondary place. The pleasant aroma and sweet or mildly sour taste of these samples must be attributed primarily to them. During summer, however, the lactobacilli were more prominent in some samples although the majority still showed either a preponderance of cocci over rods or an equal proportion of both cocci and rods. A few highly sour samples collected from Calcutta and Brindavan (Mathura) contained a majority of lactobacilli, which appeared to be different from the common types occurring in south Indian samples. These were short rods in pairs and in chains, often developing into filaments while the latter were long rods showing granules predominantly.

The yeasts seem to be inevitable contaminants of *dahi* and their greater incidence in south Indian samples may be due to their higher acidity. Most of the yeasts in *dahi* do not produce much alcohol although some are able to attack lactose as evidenced by the presence of gas in a number of samples. They are probably responsible for bringing about peptonisation and flavour defects as well as for creating favourable conditions for the growth of lactobacilli [*Joshi and Ram Ayyar*, 1936]. An appreciable number of samples obtained in Calcutta and Bombay, particularly during the monsoon, were characterised by alcoholic and estery flavours, presumably due to the action of some Saccharomyces and species of Oidia. The humid conditions in these areas are particularly favourable for the growth of these organisms. Of the other contaminants found in *dahi*, spore-forming bacteria were the most frequent ones. A number of samples showed small rods (probably some species

of Microbacterium) which formed yellow rings on the surface of *dahi*. Characteristic colonies of moulds belonging to Oidium, Cladosporium, Aspergillus, and Penicillium species as well as a few species of Actinomyces were also found in old samples. These organisms were probably dormant in the freshly made *dahi*. The greatest number of samples showing the above types of contaminants were found in Gujarat and a smaller number in south India.

The variations in fat, protein, ash, and lactose contents as well as total bacterial counts and titratable acidities of the *dahi* samples collected from different areas are shown in Table III. The figures for lactose are available for a few samples only, as its determination was discontinued for reasons explained earlier. In view of the extremely wide variations in the values, averages have not been drawn but only the ranges of variation have been given in all cases.

It is seen (Table III) that the total bacterial counts as well as the results of chemical analyses are highly variable and cannot be correlated with either seasonal or geographical conditions. This may be due to the highly variable initial quality of milk used, the type of starter added, temperature of storage, age of the samples, etc.

The total bacterial counts of the samples ranged from 13 to 22 millions per gram of *dahi* in the case of samples collected from Delhi and adjacent centres during winter while samples of a similar quality from Lahore showed counts varying from 319 to 590 millions. In the case of samples collected from Bombay, the counts ranged between 18 and 730 millions per gram of *dahi*. Since lactic acid bacteria derived from the starter constitute the predominant flora in *dahi*, the bacterial counts are influenced mainly by the types of starter organisms, temperature of storage and age of *dahi*. Due to uncertainty in regard to these factors very little reliance can be placed on the present data as an index of the quality of *dahi* or of the sanitary conditions during its production. On the other hand an estimate of the relative numbers of streptococci and lactobacilli might be of some value in determining the quality of the starter. Since the conditions in *dahi* are not favourable for the growth of coliform organisms or spore-forming bacteria, their presence in *dahi* samples may be expected to serve as an indication of unsatisfactory processing of milk, use of contaminated starter or insanitary conditions of manufacture and handling. Excessive numbers of yeasts or fungi may also be considered to be indications of poor quality of *dahi*.

The values for titratable acidity and lactose are affected to the greatest extent by microbial activity. The initial acidity of the milk is also important in assessing the significance of titratable acidity of *dahi*. A very large percentage of the samples in south India have shown acidities exceeding 1·5 per cent (expressed as lactic acid), which accounts for their highly acidic taste. This is obviously due to the preponderance of lactobacilli in the starter used, age of the sample and the high temperatures to which the product was exposed during handling. The north Indian samples on the other hand, which were generally not more than 24 hours old at the time of sampling and also contained a preponderance of streptococci, had acidity values usually below 1·5 per cent. Most of the samples associated with pleasant aroma and sweet taste showed acidities ranging from 1·0 to 1·3 per cent. The figures for lactose content are available only for a limited number of samples collected

near about Bangalore. With the exception of a few samples collected at Bangalore and examined immediately, the values for others were generally below 1 per cent and they did not also bear any uniform relationship with the corresponding titratable acidities of the samples. Although the decrease in lactose content may be attributed to the continued fermentation of lactose by lactic acid bacteria, depending on the age of the samples at the time of testing, the effect of dilution with water and formation of volatile acids from lactose by heterofermentative species, should also to be taken into consideration.

The values for fat, ash and protein contents ranged from 0·1 to 9·1 per cent, from 0·22 to 0·85 per cent and from 1·29 to 5·15 per cent respectively. Since these constituents of milk are not utilised to any appreciable extent by lactic acid bacteria, the wide fluctuations in the values, particularly in the case of fat, are primarily due to the initial quality of the raw material. All types of milk, cow, buffalo, mixed, or skimmed milks as well as milk containing added water—are used in the manufacture of *dahi*. The fat and protein contents of *dahi* samples, in which yeasts and moulds have been allowed to grow, would also be affected by the lipolytic and proteolytic activities of these organisms. The high values of protein and ash shown by a number of samples on the other hand may be partly due to the concentration of milk by heating prior to addition of starter.

TABLE IV

Results of chemical analysis of typical dahi *samples*

Area	Acidity (per cent lactic acid)	Fat per cent	Protein per cent	Ash per cent
Bangalore (W)	1·36	0·7	2·90	0·67
	0·94	1·6	3·26	0·60
	1·39	1·0	3·78	0·76
	1·10	9·0	3·63	0·70
Coimbatore (W)	0·93	4·4	4·19	0·68
	1·13	4·0	3·93	0·69
	1·58	4·4	3·54	0·66
	1·02	5·2	3·44	0·72
	1·07	8·4	2·98	0·66
Madras (W)	1·70	1·3	3·02	0·63
	1·68	1·2	3·02	0·53
Calcutta (S)	0·81	2·8	3·29	0·71
	1·11	4·0	3·67	0·69

TABLE IV—*contd.*

Results of chemical analysis of typical dahi *samples*—contd.

Area	Acidity (per cent lactic acid),	Fat per cent	Protein per cent	Ash per cent
	1·05	4·4	3·99	0·64
	1·36	5·6	3·82	0·73
Bombay (W)	1·66	3·2	4·05	0·64
	1·86	6·2	4·19	0·68
	1·32	8·4	4·06	0·68
Bombay (S)	1·98	5·0	4·75	0·63
	0·87	6·4	4·56	0·67
	1·03	6·8	3·85	0·63
	2·45	6·8	4·62	0·70
	1·37	7·4	4·56	0·71
Allahabad (W)	..	4·6	4·43	0·67
	..	5·0	3·92	0·71
Allahabad (S)	1·39	1·4	3·92	0·66
	1·31	4·4	4·14	0·68
	..	4·8	4·51	0·63
	1·19	6·6	3·67	0·59
	..	8·4	3·99	0·63
Delhi (W)	..	5·4	4·24	0·68
	..	4·6	4·32	0·61
Delhi (S)	1·49	6·0	4·55	0·73
	1·39	..	3·90	0·60
Karnal (W)	..	6·4	3·87	0·70
	..	8·6	3·74	0·70
Cuttack (S)	1·50	2·8	3·35	0·67
	1·09	3·6	3·85	0·70
	1·08	4·6	3·15	0·63

(W) Winter. (S) Summer.

Among the constituents of *dahi*, ash content appeared to show the least variation due to the type of milk used. In Table IV, the individual values for acidity, fat, protein and ash in respect of a few typical samples of *dahi*, which might be considered to be fairly normal on the basis of their ash contents, have been shown. The results bring out the variations which may be expected in the composition of market *dahi* samples and agree closely with the values recorded by Davies [1940] for different types of *dahi*. The samples of *dahi* with low fat content may be presumed to have been prepared from skimmed milk or a mixture of whole and skimmed milks and those having high fat content as having been made from buffalo milk. The variations in the methods of preparation, particularly the extent of concentration of milk, have considerable influence on the values. Accordingly, the present data are useful only in providing information on the quality of *dahi* available in the market and cannot serve as a basis for fixing any chemical standards for this product. It would be desirable to collect further information on the normal variations in the composition of *dahi* with reference to the type of milk used, age of samples and other conditions of production.

Summary

A qualitative survey of the types of *dahi* produced and marketed in different parts of India has been made. A total number of nearly 1,000 samples of *dahi* (including *lassi*) were collected from dairies, *halwais*, street vendors and private houses in different localities. They were examined for texture and flavour, titratable acidity, nature of microflora (as revealed by microscopic appearance), total bacterial count and percentages of fat, protein and ash.

Samples of *dahi* collected from North India were generally characterised by firm curd formation and sweet to mildly sour taste with an agreeable flavour, although during summer and monsoon an appreciable number of them were sour and also showed gassiness and peptonization. Some of the best samples were obtained from Tirhut Valley (Bihar), Allahabad, Karnal, Brindavan (Mathura) and Calcutta.

Samples from south India were usually poor or watery in texture and sour to taste, irrespective of the season, while a large percentage of samples secured from sea coast areas, particularly during monsoon, showed gas formation, alcoholic and estery flavour as well as mould growth.

There was either a preponderance of streptococci or a balanced proportion of streptococci and lactobacilli (short rods in pairs or chains) in the samples of *dahi* produced in north India. Yeasts were present in many cases and during monsoon both yeasts and lactobacilli were more numerous. Almost all south Indian samples of *dahi* contained a preponderance of lactobacilli (large rods with prominent granules) as well as yeasts, while streptococci were present in samples collected in Gujarat, Nagpur and Bombay.

The above differences between the samples from different areas have been attributed to the initial quality of milk, type of starter used, technique of manufacture and influence of atmospheric temperature.

There were very wide variations in the values for acidity, fat, protein and ash irrespective of the source or season of collection. The significance of the initial

quality of milk and other factors in relation to the quality of *dahi* has been pointed out.

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STUDIES ON *DAHI**

III. TAXONOMY OF THE LACTIC ACID BACTERIA OF *DAHI*

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(With Plates I—III)

A BRIEF review of literature on the microbiology of fermented milks including *dahi* has been given in Part I of the present series of papers [Laxminarayana and Iya, 1952]. There is, however, very little information regarding the organisms associated with *dahi* and except for the isolation of a lactobacillus species—*Streptothrix dadhi* [Chatterjee, 1910, and Ram Ayyar, 1928]—and two species of streptococci, *S. Lactis aromaticus* [Joshi and Ram Ayyar, 1936] and *S. diacetyl aromaticus* [Karnad, 1939]. No systematic study of this important subject has been made. From a microscopic examination of *dahi* samples, collected from different parts of India [cf. Part II, Laxminarayana *et al.*, 1952] it was observed that South Indian samples generally showed a predominance of lactobacilli while North Indian varieties contained a greater proportion of streptococci. The types of lactobacilli occurring in the latter samples also appeared to be mostly short rods in pairs, chains or filamentous forms. The results of further studies carried out on the isolation, characterization and taxonomical classification of the lactic acid organisms, found in different samples of *dahi*, are reported in this paper.

The isolation of pure cultures of lactic acid bacteria from *dahi* or other material does not present much difficulty because these organisms as a group are easily distinguished from other bacteria by their morphology, cultural characteristics and fermentation reactions [Davis, 1936 ; Orla Jensen, 1942 ; Breed, Murray and Hitchens, 1948] and suitable methods, based on their nutritional and other requirements, are also now available for their cultivation [Orla Jensen 1942 ; Levine and Schoenlein 1930 ; Davis, 1935a, 1939 ; *Manual of Methods*, 1944 ; Tanner, 1944]. The taxonomical classification of lactic acid organisms is, however, recognized to be very complicated due to the overlapping of characteristics of different species and variations encountered within the same species. In addition, there has also been considerable confusion in the nomenclature of lactic acid bacteria adopted by different workers. Orla Jensen [1919 and 1942], who was the first to make a systematic study of lactic acid bacteria associated with dairy products, emphasised mainly growth temperatures, nature of fermentation products and acid producing

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capacity of the species in distinguishing one from the other and considered that sugar fermentations, morphology and cultural characteristics were of secondary importance. His grouping of the organisms into homofermentative and heterofermentative types and of the homofermentative lactobacilli into thermobacteria and streptobacteria is based on such considerations. Although this scheme has been generally accepted by later workers, the differentiation of closely allied species of organisms has been a controversial topic.

One of the most classical controversies has been with regard to the identification of *L. bulgaricus* and *L. acidophilus*. Several workers have attempted to differentiate between the two organisms on the basis of sugar fermentations [Kulp and Rettger, 1924], inhibition by indol and phenol [Kulp, 1926] and the effect of surface tension depressants [Albus and Holm, 1926; Kopeloff and Beerman, 1925]. These tests were reported to be unreliable by Day and Gibbs [1928]. Kulp and Rettger [1924] stated that the ability of *L. acidophilus* to survive in the intestines when the culture was fed to laboratory animals was its most important distinguishing feature since cultures of *L. bulgaricus* could not be implanted in the intestines. Curran, Rogers and Whittier [1933] employed growth temperatures, colony appearance, fermentation of raffinose and mannitol, optical rotation of the acid produced and tolerance to phenol and indol, while Sherman and Hodge [1940] used salt tolerance, influence of *p*H, growth temperature and fermentation of sugars as criteria for differentiating between the two organisms as also for separating other species of lactobacilli. Lazicova [1939] was of the opinion that both the organisms belonged to the same species and any differences observed between the two were only due to strain variations. On the other hand, *L. acidophilus* and *L. casei* were considered to be R and S forms of a single type by Pederson [1947], who found that the strains of *L. acidophilus*, originally described as forming round and filamentous colonies and producing inactive acid [Curran, Rogers and Whittier, 1933], had changed into smooth forms producing dextro-rotatory acid and showing other typical characteristics of *L. casei*. The former is an intestinal organism while the latter is always associated with milk and milk products and the gradual transformation in the characteristics of *L. acidophilus* may be attributed to dissociation of the original R strains into R and S forms and the subsequent preponderant development of the S forms during continued maintenance in milk. The reversal of this process has not been reported by any worker so far. Similar variations in characteristics due to dissociation of the organisms have been observed [Okulitch, 1939; Barber and Frazier, 1945]. The distinctive features which characterise *L. casei*, *L. plantarum* and heterofermentative lactobacilli found in cheese have been described by Davis [1935] and Sherwood [1939]. The former investigator, stressing the importance of ecological aspects in characterising the organisms, suggested that they would influence the preference of the organisms to grow in a particular medium or to ferment a particular sugar especially in the case of freshly isolated strains since on prolonged cultivation in the laboratory, the natural properties of the organisms are likely to undergo some variation. A detailed description of various species of lactobacilli has also been given by Holland [1920], Fred, Peterson and Anderson [1921 and 1922] and Pederson [1936 and 1938]. Recently, Tittsler, *et al.* [1947] has attempted to classify the lactobacilli on the basis of their colony formation, optical activity of lactic acid produced, maximum acidity produced, growth temperature and ability to produce gas.

The identification of streptococci also has been somewhat confused particularly in the distinction between *S. lactis* and *S. cremoris* and between *S. lactis* and *S. faecalis*. *S. cremoris* was for a long time considered to be a variant of *S. lactis* but on the basis of the differences in their maximum growth temperatures, their tolerance of alkali and salt in the medium, their inhibition by methylene blue and their capacity to produce ammonia from peptone, they are now generally accepted as two distinct species [Yawger and Sherman, 1937]. Davis [1935, b] has drawn attention to the differences between the two species in respect of morphology, inhibition by lactate, and fermentation of maltose, dextrin, arabinose and mannite, most of which have been confirmed by Hunter [1946]. Shattock and Mattick [1943] found a close serological relationship between the two species although their phage reactions were different. Similarly, *S. faecalis* has been differentiated from *S. lactis* on the basis of its higher heat resistance as well as alkali and salt tolerance [Sherman and Stark, 1934]. Sherman *et al.* [1940] found *S. lactis* and *S. faecalis* to be serologically distinct, while Gunsalus *et al.* [1944] reported that *S. lactis* was a variant of *S. faecalis*. Sherman [1937] in his critical review of the literature on the taxonomy of streptococci pointed out certain significant differences in the cultural and physiological properties of these organisms which could be utilised for diagnostic purposes.

The heterofermentative streptococci (genus leuconostoc) and the streptococci commonly employed in butter cultures have been reviewed by Hucker and Pederson [1930] and by Hammer and Babel [1943]. The Enterococci and related streptococci were studied by Sherman [1938]. In the classification of enterococci as well as their differentiation from lactic streptococci both physiological and serological reactions have been employed [Sherman, 1938 ; Graham and Bartley, 1939 ; Shattock and Mattick, 1943 ; Shattock, 1945 ; Evans and Chinn, 1947]. Bacteriophage specificity is another criterion that has been used for the differentiation of *S. lactis*, *S. cremoris* and *S. faecalis* [Shattock and Mattick, 1943 ; Hunter, 1946], but this test would appear to hold promise only for those species for which the specific phages have been isolated. It is also known that the bacteriophages are highly influenced by strain variations and other factors. Among the other characteristics, which have been used for distinguishing the different species of streptococci, may be mentioned the following: behaviour towards various oxidation-reduction indicators [Davis, 1938]; influence of *p*H [Davis and Thiel, 1939]; utilisation of lactate and citrate as sources of carbon [Foster *et al.*, 1942 ; Campbel and Gunsalus, 1944] ; production of ammonia from arginine [Niven *et al.*, 1942] and production of carbon dioxide from glucose [Gibson and Abd-El-Malek, 1945]. Exceptional strains of *S. thermophilus*, *S. bovis* and other species which gave variable results in respect of some of the above tests have been reported by various workers [Wright, 1936 ; Nichols, 1939 ; Nichols and Hoyle, 1948]. In a recent study of the streptococci of milk, Abd-El-Malek and Gibson [1948] have outlined the most typical taxonomic characteristics of the different groups of streptococci.

Davis and Rogers [1939 a and b] grouped different species of streptococci and lactobacilli on the basis of their metabolic activities and later Davis [1940] outlined a broad scheme for simplifying the classification of the organisms on the above basis. Kitahara [1940] suggested a new classification of lactic acid bacteria based on morphology, sugar fermentations and other properties. In the Sixth Edition of Bergey's Manual [Breed, Murray and Hitchens, 1948] an attempt has been made

to base the classification of the species on the most consistent and easily differentiable characteristics of the organisms reported by different workers and to reduce the number of independent species by assigning species rank only to such organisms which have been described fully and whose characteristics have been well established. In recent years some workers have suggested the classification of lactic acid bacteria on the basis of their specific nutritional requirements [Hucker *et al.*, 1944; Dunn *et al.*, 1947; Hoff-Jorgensen *et al.*, 1947]. Although a knowledge of the vitamin and amino acid requirements may help in differentiating species which are closely related to each other, there is a possibility of encountering several anamolous results by this method. For example, *L. delbruckii* and *L. casei*, which are quite different from each other in their physiological as well as biochemical reactions, were considered to be no more than varieties of the same species because their nutritional requirements were similar [Dunn *et al.*, 1947]. The former is a non-lactose fermenting organism occurring in molasses and growing at high temperatures while the latter is a lactose fermenting lactobacillus commonly found in dairy products and able to grow only at temperatures below 45°C. The nutritional requirements of an organism can, therefore, be considered useful only in providing supplementary evidence regarding its identity. It would thus appear that the taxonomical classification of lactic acid bacteria will have to be based only on such cultural, and physiological characteristics as have been considered to be stable and typical of the groups or species under question, having regard to the source from which the isolates were obtained and the conditions of their subsequent maintenance.

A summary of the typical, well-established characteristics of different species of lactic acid bacteria which have been described by various workers [Orla Jensen, 1942; Sherman, 1937; Breed *et al.*, 1948] and which have been considered to be important in the taxonomical classification of the organisms in this study is given in Table I.

Experimental

Isolation of lactic acid bacteria

The original samples of *dahi* as well as stab cultures of the organisms made from *dahi* at various centres, secured in the manner explained in Part II [Laxminarayana *et al.*, 1952] of this series, were used for the isolation of lactic acid bacteria.

As soon as the samples were received, a microscopic examination was carried out to determine the predominant types of organisms present. Small amounts of well mixed samples were then transferred to sterile yeast dextrose litmus milk tubes. One set of tubes was incubated at 45°C., one at 37°C., and a third set at room temperature (24° to 30°C.). When the inoculated samples had coagulated or shown acid production, they were plated either immediately or after further transfers in order to eliminate yeasts and encourage the growth of specific types of lactic acid organisms. Immediate plating gave only the most active types of rods or cocci present in *dahi*. Repeated transfers resulted in obtaining pure cultures of organisms having their optimum near the respective temperatures of incubation. Rapid transfers of the cultures soon after curdling were helpful in securing a predominance of streptococci over lactobacilli.

TABLE I (a)

Summary of characteristics used for the classification of lactic acid bacteria

STREPTOCOCCI

	Name of Species	Growth temperature °C			Action on litmus milk	Maximum acidity in milk (Percentage lactic acid)	Volatile acidity in milk	CO_2 from glucose	NH_3 from arginine	Acetoin from citrate	Tolerance to methylene blue	NaCl Tolerance			pH Tolerance		Fermentation of		Heat resistance (63°C. for ½ hour)	Remarks
		10	45	50								2 per cent	4 per cent	6·5 per cent	9·2	9·6	Sucrose	Maltose		
I.	*S. thermophilus*	0	+	+	AC*	1·00	0	0	0	0	0	0	0	0	0	0	+	0	+	Milk or lactose media.
	S. bovis	0	+	0	Ar or AC	0·60	0	0	0	0	0	+	0	0	0	0	+	+	+	Grows slowly in milk.
II.	*S. lactis*	+	0	0	ARC	0·95	0	0	+	0	+	+	+	0	+	0	±	+	0	Mostly diplococci; grows at 40°C; not inhibited by 0·3 per cent methylene blue.
	S. cremoris	+	0	0	ARC	0·70	0	0	0	0	+	+	0	0	0	0	0	0	0	Usually long chains no growth at 40°C. Inhibited by 0·3 per cent methylene blue.
III.	*S. faecalis*	+	+	±	ARC	0·80	0	0	+	+	+	+	+	+	+	+	+	+	+	—
	S. liquefaciens	+	+	±	ARCP	0·95	±	0	+	+	+	+	+	+	+	+	+	+	+	Gelatine liquefied.
	S. durans	+	+	+	AC	..	0	0	+	+	+	+	+	+	+	+	0	+	+	Many strains form hemolytic colonies.
IV	*S. dextranicum* (*S. paracitrovorus* or *S. kefir*).	+	0	0	A or AC (g)	0·27 0·90	+	+	0	0	..	..	..	..	..	..	+	+	±	Grows at 37°C. very slow growth in milk; acetoin produced in milk; stimulated by yeast extract.
	L. citrovorum (*S. citrovorus*).	+	0	0	a (g)	slight	+	+	0	0	..	..	..	..	..	..	0	0	0	No growth at 37°C. veryslow growth in milk. Acetoin produced in milk; stimulated by yeast extract.

*The order in which the letters are placed indicate the chronological sequence of changes.
A = Acid; a = Slight acid; C = Clot; R = Reduction of litmus; r = Slight reduction of litmus; P = Proteolysis of milk; g = Gas production; ± = doubtful or slight; o = Negative; + = Positive.

TABLE I (b)

Summary of characteristics used for the classification of lactic acid bacteria

LACTOBACILLI

Group.	Name of Species	Growth temperature °C.					Action on litmus milk	Time of curdling milk	Maximum acidity in milk (percentage of lactic acid)	Volatile acidity + CO_2 in milk	Growth in 2 per cent lactate media	Tolerance to methylene blue (0·1 per cent)	NaCl tolerance		Fermentation of		Heat resistance 63°C. for ½ hour	Remarks
		10	15	22	45	50							2·5 per cent	5 per cent	Sucrose	Maltose		
I.	*L. bulgaricus*	0	0	+	+	+	AC*	24	>2·0	0	0	+	0	0	+	0	+	Inhibited by 0·5 per cent bile salt.
	L. caucasicum	0	0	0	+	0	AC	24	>2·0	0	0	0	..	.	+	+	+	Best growth with yeast.
	L. lactis	0	0	+	+	+	ARC	24-48	1·4-1·8	0	0	0	+	0	+	+	+	—
	L. helveticus (*L. casei E*).	0	0	+	+	±	ARC	24-72	>2·0	0	0	+	+	0	0	+	+	—
	L. acidophilus	0	+	+	+	0	ARC	24-72	>2·0	Traces	0	+	+	0	+	+	+	Not inhibited by 0·5 per cent bile salt.
II.	*L. casei* (*L. casei L*)	+	+	+	±	0	ARC	2-6 days	1·4-1·8	0	±	0	+	+	+	+	0	Prefers lactose to Maltose.
	L. plantarum (*L. arabinosus*).	+	+	+	0	0	ARC	7-14 days	1·4-1·8	Slight	0	..	+	+	+	+	±	Stimulated by yeast; prefers maltose to lactose.
III.	*L. brevis*	+	+	+	±	0	A or AC (g)	15-30 days	<1·0	High	+	0	+	+	+	+	+	Marked stimulation by yeast extract; marked fermentation of pentose (arabinose).
	L. fermenti	0	+	+	+	±	AR (g)	OC	<1·0	High	—	..	—	+	+	+	+	Stimulated by yeast extract; weak or no fermentation of pentoses.

*The order in which the letters are placed indicate the chronological sequence of changes.
+ = Positive; ± = variable or a few strains positive; 0 = Negative; A = Acid; C = Coagulation; P = [illegible] of curd; R = Reduction of litmus before clot; g = May show gas formation; a = slight acid; r = slight reduction.

When agar or purple lactose agar, fortified with one per cent yeast autolysate, was generally employed for pouring plates. Almost all organisms grew well on these media and formed clear colonies which were easy for picking. In later series of samples, yeast dextrose agar containing one per cent sodium citrate was employed for encouraging the growth of heterofermentative streptococci while tomato juice agar or wort agar was used for cultivating heterofermentative types of lactobacilli. The various media were prepared according to standard methods [Levine and Schoenlein, 1930 and Tanner, 1944]. The plates were incubated at 30°C., 37°C., and 45°C., for 3 to 4 days or longer until well developed colonies appeared. Small, discreet colonies with entire or irregular margins growing below the surface and showing acid production were recognized to be lactic acid organisms. Under the low power (x 40) of a compound microscope they appeared either as smooth, rough or filamentous colonies.

Well isolated representative colonies from each of the plates were picked into yeast dextrose litmus milk tubes and incubated at temperatures at which the colonies had been cultivated. After the cultures showed evidence of growth they were examined microscopically and purified by further plating and repeated sub-culturing in milk. In all cases, the organisms were tested for catalase production (recognized by the evolution of gas bubbles when 1 per cent solution of hydrogen peroxide was added on to the agar colonies) in order to eliminate the catalase positive micrococci and other contaminants which might be mistaken for lactic acid bacteria. Only such cultures as were found to be catalase negative and as did not show appreciable surface growth on agar slants were presumed to be lactic acid bacteria [Orla Jensen, 1942] and taken up for further observations.

A total of 720 isolates, including 290 streptococci and 430 lactobacilli, were obtained in the above manner. The isolates were maintained in milk at a low temperature (10° to 15°C.) and transferred once every ten days.

Classification and identification of the organisms

As the number of isolates to be examined was very large it was found convenient first to make a preliminary classification of the organisms based on well-recognized group characteristics and then select a limited number of representative strains for further study and identification.

Preliminary grouping of the organisms

All the isolates were subjected to the following tests for their preliminary classification.

Microscopic appearance. Microscopic appearance and arrangement of the cells of organisms in freshly curdled milk cultures were examined.

Action on litmus milk. The cultures (soon after curdling) were inoculated into plain litmus milk and incubated at 37°C. Observations were made regarding acid production, clotting, reduction of litmus, gas production and proteolysis.

Growth at different temperatures. Young cultures of the organisms were inoculated into plain litmus milk and yeast litmus milk and incubated at 10°C., 30°C., 37°C., 45°C., and 50°C. The rate of acid production (indicated by colour of litmus) and he time of curdling were noted.

Influence of yeast extract. The influence of yeast extract on the growth of the organisms was assessed by observing the relative time-intervals of curdling in plain and yeast litmus milk.

Aroma production. The development of typical aroma in the above milk cultures at 37°C. was tested organoleptically. Production of acetyl methyl carbinol and diacetyl was also examined by the creatine test [Hammer, 1935].

Fermentation of selected sugars. The cultures were inoculated into yeast peptone broth containing one per cent sugar and brom cresol purple as indicator and incubated at 37°C. Production of acid and gas in dextrose, lactose, sucrose and maltose broths was observed. Gas formation could not be observed distinctly in the sugar broths although a few cultures showed evidence of this in milk cultures.

Detailed examination of selected organisms and their identification

After a preliminary classification of the isolates was made (Table II) a selected number of organisms in each of the different groups of streptococci and lactobacilli were taken up for further study. In making the selection, attempts were made to include as far as possible organisms isolated from representative samples of *dahi* collected in different centres in the country particularly those from areas reputed for their industry.

The morphological, cultural and biochemical characteristics of these organisms were determined by the methods recommended in the *Manual of methods for Pure Culture Study of Bacteria* [1944], except for some minor modifications. Some of the specific tests for confirming the identity of the organisms were carried out according to the procedures suggested by previous workers. All the reactions were studied at 37°C., unless otherwise stated.

(i) *Morphology*

(*a*) Form, size and arrangement of cells in freshly curdled milk culture by a microscopic examination of the smear, defatted with xylene, fixed in alcohol and stained with borax methylene blue.

(*b*) Gram staining and spore formation observed in 24 hour old agar cultures.

(*c*) Motility in 24 hour old dextrose broth cultures.

(ii) *Temperature relations*

(*a*) Ability to grow in milk at 10, 15, 22, 45 and 50°C.

(*b*) Resistance to pasteurisation temperature (63°C. for 30 minutes).

(iii) *Cultural characteristics*

(*a*) Colony formation on lactose or dextrose agar plates.

(*b*) Growth on lactose agar slant.

(*c*) Growth in dextrose or lactose agar stab.

(iv) *Growth in milk*

(*a*) Action on litmus milk (acid, clot, reduction of litmus and proteolysis)

(*b*) Time of coagulation of milk at 37°C.

(*c*) Maximum titratable acidity (percentage of lactic acid) produced in milk. after 7 to 10 days incubation at 37°C.

(v) *Chemical tolerance* [Sherman, 1937].

(*a*) Alkali tolerance of streptococci (growth in yeast dextrose broth adjusted to *p*H 9·2 and 9·6).

(*b*) Salt tolerance (growth in yeast dextrose broth containing 2 per cent 4 per cent and 6 per cent NaCl in case of streptococci and 2·5 per cent and 5 per cent NaCl for lactobacilli).

(*c*) Methylene blue tolerance (growth and reduction of methylene blue in yeast dextrose broth containing 0·1 per cent methylene blue).

(vi) *Biochemical reactions*

(*a*) Fermentation of important sugars, alcohols and starch (production of acid and/or gas in yeast peptone broth containing one per cent of the fermentable carbohydrate and brom cresol purple as indicator); Changes observed upto 20 days.

(*b*) Utilization of lactate as a source of carbon in yeast peptone broth containing two per cent sodium lactate [Foster *et al.*, 1942]. } Lactobacilli only.

(*c*) Acetoin production from citrate in glucose free media [Abd-El-Malek and Gibson, 1948] for Streptococci only.

(*d*) Production of carbon dioxide from glucose in semi-solid agar medium [Gibson and Abd-El-Malek, 1945].

(*e*) Reduction of nitrate to nitrite.

(*f*) Indol production in peptone broth.

(*g*) Ammonia production from arginine [Niven *et al.*, 1942] for streptococci only.

(*h*) Volatile acid production in milk cultures [Hammer, 1948].

Results and discussion

Preliminary classification of the organisms

Altogether 290 streptococci and 430 lactobacilli were isolated from over 500 samples of *dahi* [Laxminarayana *et al.* 1952]. They were found to form small, discreet colonies on lactose agar plate and under the low power of the microscope the colonies were observed to be round or oval, ranging from smooth to rough varieties. Surface growth on dextrose or lactose agar slant was very poor and the organisms grew well in agar stabs indicating their micro-aerophilic character. There was no evidence of catalase production and all the isolates were Gram positive without showing any endospore formation or motility.

On the basis of the preliminary tests the isolates could be classified into the following distinct groups with well defined characteristics (Table II).

Table II

Preliminary classification of lactic acid bacteria isolated from dahi

Group	Number of isolates	Growth at 10 °C.	Growth at 45 °C.	Growth at 50 °C.	Action on litmus milk†	Time of coagulation of milk at 30	Time of coagulation of milk at 37	Time of coagulation of milk at 50	Stimulation of activity by yeast extract	Aroma and acetion production	Fermentation of sugars*: Dextrose	Lactose	Sucrose	Maltose	Remarks
						hours	hours	hours							
STREPTOCOCCI I. *S. thermophilus*	140	0	+	±	AC	24-96	8-24	4½-12	0	0	+(12)	++	++	0	
II. *S. lactis*	28	+	0	0	ARC	8-24	8-24	—	0	0	+	+	0(14)	+(3)	
III. *S. faecalis*	107	+	+	0	ARC[a]	24-48	8-24	—	±	±	+	—	0(53)	+(17)	a=9 isolates caused rapid proteolysis of milk
IV. *S. dextranicum*	15	+	0	0	ARC	3-10 days	3-10 days	—	++	++	+	+	+(3)	0(4)	
LACTOBACILLI 1. *L. bulgaricus* (A)	109	0	+	+	AC	18-48	9-24	5-10	±	0	++	++	0	0	
(B)	102	0	+	0	AC	24-48	12-24	—	±	0	++	++	0(20)	0(20)	
(C)	46	0	+	+	ARC	24-72	24-48	16-24	0	0	+	+	+(11)	+(11)	
(D)	53	0	+	+[b]	ARC	48-96	24-72	24-48	±	0	+	+	0(10)	+	b=21 isolates not able to grow at 50°C.
II. *L. casei*	58	+	0[c]	0	ARC	2-14 days	2-14 days	—	+	±	+	+	+(10)	+(8)	c=18 isolates showed slight growth at 45°C.
III. *L. brevis*	22	+d	0[d]	0	A or AC	3-15[e] days	3-15[e] days	—	++	++	+	+	+	+	d=10 isolates did not grow at 10°C. but showed growth at 45°C. e=12 isolates showed only slight acid even after a month. They also showed slight gas formation in milk.

* Figures in brackets indicate number of isolates which were exceptions.
R=Reduction of litmus; r=Partial reduction of litmus;
± =Doubtful or variable; 0=Negative.
A=Acid; C=Coagulation;
+ =Positive; ++=Highly positive or rapid;
†The order in which the letters are placed indicate the chronological sequence of changes.

Streptococci :

S. thermophilus	group	(140 isolates)
S. lactis	,,	(28 ,,)
S. faecalis	,,	(107 ,,)
S. dextranicum	,,	(15 ,,)

Lactobacilli :

L. bulgaricus	,,	(310 ,,)
L. casei	,,	(88 ,,)
L. brevis	,,	(32 ,,)

It will be seen from Table II that the streptococci included under *S. thermophilus*, *S. faecalis* and *S. lactis* groups were all able to grow rapidly in milk, producing acid and coagulating milk within 24 hours at 37°C. The organisms in *S. thermophilus* group were further characterised by their ability to grow at 50°C. (coagulating milk in 4½ to 12 hours), inability to reduce litmus and their ability to ferment lactose and sucrose vigorously. Some of them showed unusually weak fermentation of dextrose. The isolates in the *S. lactis* and *S. faecalis* groups showed growth at 10°C. while the latter were able to grow at 45°C. also. Both were able to reduce litmus and generally fermented maltose but rarely sucrose. Appreciable development of aroma and acetoin production were noticed in milk cultures of a few isolates while seven organisms resembled *S. liquefaciens* in their ability to proteolyse milk.

The isolates in the last group (*S. dextranicum*) were generally slow-growing types taking three to five days or even longer for coagulating milk. Their activity was considerably stimulated by yeast extract. They showed a marked development of diacetyl aroma in milk cultures.

The organisms in *S. thermophilus* and *S. faecalis* groups appeared to be the most predominant types of cocci occurring in *dahi* of almost all parts of the country. The *S. lactis* group of organisms were obtained mostly from samples of *dahi* collected in Calcutta, Patna, Delhi and other places in north India during winter. The heterofermentative types of streptococci occurred mostly in samples collected from Patna, Banaras and Allahabad which are famous for their *dahi*.

In regard to the lactobacilli, all the isolates growing at 45°C., but not at 10°C., and showing rapid acid production in milk have been included in Group I under *L. bulgaricus* group (Thermobacteria). With the exception of the organisms in sub-group D, they generally appeared as long and slender rods, occurring singly (occasionally in long filaments) and showing metachromatic granules prominently. The isolates in subgroup A resembled the typical *L. bulgaricus* species in their ability to grow at 50°C., their rapid acid production in milk (coagulating it in 5 to 10 hours at 50°C. and in 9 to 22 hours at 37°C.) without reducing litmus, their vigorous fermentation of lactose and their inability to ferment sucrose or maltose. The organisms in sub-group B differed from the above mainly in their inability to grow at 50°C. and some of them were also able to ferment sucrose. The other two subgroups included organisms which were generally able to grow at 50°C., reduce litmus and coagulate milk in one to three days. Most of them fermented maltose and organizms in sub-group C fermented sucrose also.

The isolates in *L. casei* group (Streptobacteria) appeared as short and thin rods occurring in pairs or short chains and showing granules occasionally. They showed growth at 10°C. and a few isolates grew at 45°C. also. Nearly 50 per cent of them were able to coagulate milk in 2 to 3 days at 37°C. while the rest took 10 to 14 days. Litmus was partially reduced and both maltose and sucrose were generally fermented.

The remaining lactobacilli, which were characteristically slow acid producers have been included in *L. brevis* group. They generally resembled the isolates in *L. casei* group in their morphology. With the exception of a few organisms they did not grow at 45°C. but showed slow growth at 10°C. All the organisms fermented dextrose, lactose, sucrose and maltose. Half of them produced only slight acid in milk even after a month but in presence of yeast extract milk was observed to coagulate in 3 to 6 days. Slight gas production was observed in milk cultures of some of these isolates. The remaining 50 per cent of the isolates in this group were able to coagulate milk in 3 to 10 days and also showed high aroma and acetoin formation.

The lactobacilli in *L. bulgaricus* group, particularly those included in sub-groups A and B of this group were found to occur in *dahi* produced in almost all parts of the country and these also formed the predominant flora of south Indian *dahi* samples. The organisms in *L. casei* group were found to be the predominant types of lactobacilli, occurring in north Indian samples of *dahi*, while the heterofermentative types included in *L. brevis* group were found mostly in good samples of *dahi* obtained from Calcutta, Darbhanga (Bihar) and Mathura (Uttar Pradesh).

Thus, almost all the types of lactic acid bacteria, reported to be present in fermented milks [Corminboeuf, 1933; Tanner, 1944] were observed to occur in *dahi*. The heterofermentative streptococci described under *S. dextranicum*, group-IV, did not show any evidence of their ability to produce gas either in milk or in dextrose broth cultures in subsequent experiments. They also became more vigorous acid producers on repeated sub-culturing. These astrains were accordingly considered to be heterofermentative variants of the organisms in *S. faecalis* group. Similarly, in the case of the heterofermentative types of lactobacilli included under *L. brevis*, group III only 12 isolates gave evidence of gas formation. The rest of the organisms were, therefore, considered to be heterofermentative variants of the organisms in Group II.

Detailed examination of selected isolates and their identification

The results of detailed taxonomical studies on selected isolates (32 streptococci and 21 lactobacilli) are presented in Tables III (a and b) and IV (a and b). The organisms have been grouped according to the species with which they have been identified on the basis of their typical morphological, cultural and biochemical characteristics (*vide* Table I). Photomicrographs of some of the organisms are shown in Plates I, II and III.

Certain common characteristics, associated with lactic acid bacteria as a group were observed for all the organisms. They were all Gram positive, non-motile

PHOTO MICROGRAPHS OF LACTIC ACID BACTERIA ISOLATED FROM *DAHI*.

FIG. 1. *Streptococcus thermophilus* (S. 197); Methylene blue

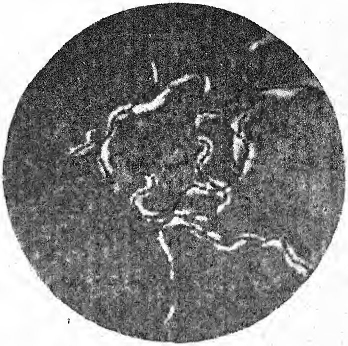

FIG. 2. *Streptococcus thermophilus* (S. 197); India ink and Methylene blue preparation showing capsules

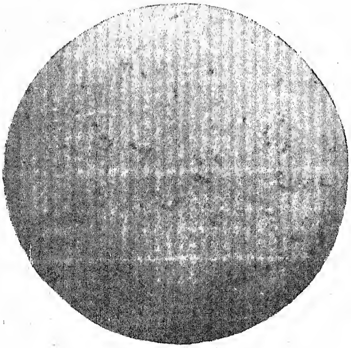

FIG. 3. *Streptococcus lactis* (S. 69); Methylene blue

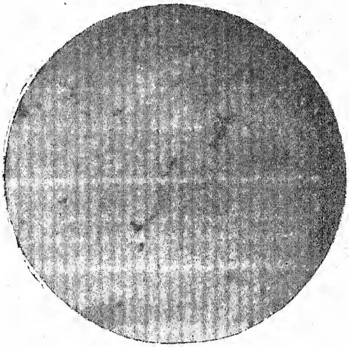

FIG. 4. *St eptococcus faecalis* ? (S. 209); Gram stain

(All photographs are of organism magnified 950 times and are of preparations of milk cultures incubated for 24 to 48 hours at the optimum temperatures of the organisms, unless otherwise stated)

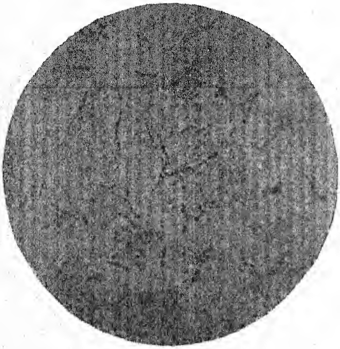

FIG. 5. *Lactobacillus bulgaricus* (L. 96); Methylene blue

FIG. 6. *Lactoba illus bulgaricus* (L. 86); 37°C. Methylene blue

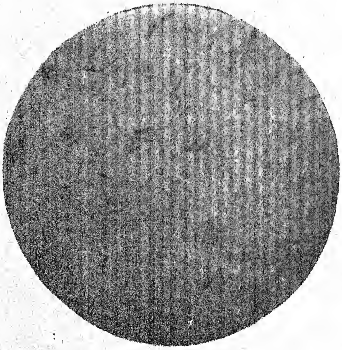

FIG. 7. *Lactobacillus lactis* (L. 326); Methylene blue

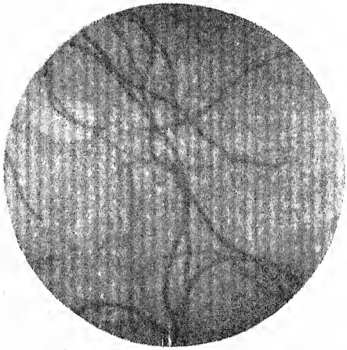

FIG. 8. *Lactobacillus helveticus* (L. 189); Methylene blue

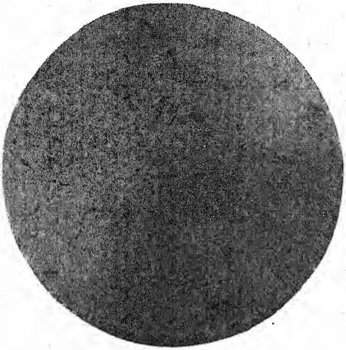

FIG. 9. *Lactobacillus cassi* (L. 134); Dextrose broth; Gram stain

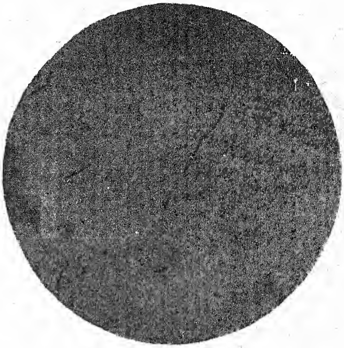

FIG. 10. *Lactobacillus plantarum* ? (L. 89) Gram stain

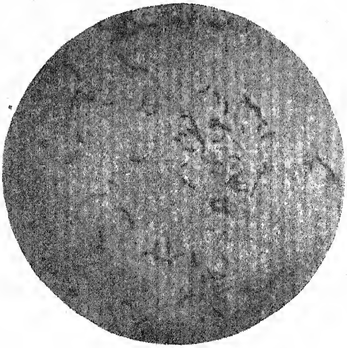

FIG. 11. *Lactobacillus plantarum* (L. 89); Dextrose broth; Methylene blue stained showing granules

FIG. 12. *Lactobacillus plantarum* (L. 89); Crystal violet stained showing capsules.

and non-spore forming organising and their colonies (deep or sub-surface) on agar were small, compact and round, oval or disc shaped. Under the low power of a microscope, the colonies of streptococci generally appeared smooth and round or oval shaped with entire margins, while in the case of lactobacilli both smooth as well as rough and filamentous types of colonies were encountered. Growth on agar slants was very scanty while good growth (filiform or beaded) was observed in a dextrose or lactose agar stab. None of the organisms was found to produce catalase, reduce nitrate to nitrite or produce indol.

Streptococci. It will be seen (Table III *a* and *b*) that the streptococci have been divided into four distinct species *viz.*, *S. thermophilus* (10 isolates); *S. lactis* (6 isolates); *S. faecalis* (10 isolates); *S. liquefaciens* (3 isolates) and heterofermentative types tentatively indentified as *S. faecalis* (5 isolates). It was not possible to differentiate between the streptococci on the basis of their morphology or colony characteristics since all of them generally appeared as spherical or oval cells (0·6 to 1·0μ diameter) arranged in pairs or short chains and formed smooth colonies. Long chains of cells and capsulated forms were conspicuously shown only by two strains of *S. thermophilus* (S. 240 and S. 197) and one of *S. lactis* (S. 10). Significant differences were, however, observed in respect of growth at different temperatures, action on litmus milk, production of volatile acid, acetoin formation from citrate and production of ammonia from arginine, which were of diagnostic value in the identification of their species. With the exception of the isolates included under *S. liquefaciens* and heterofermentative members of *S. faecalis* groups, there was no appreciable production of volatile acidity in milk. None of the streptococci studied gave evidence of gas formation either in milk, in sugar broths or in semi-solid glucose agar media. All of them fermented dextrose (except for two isolates of *S. thermophilus*) and lactose but in regard to fermentation of other sugars considerable variations were encountered even in the case of organisms included in the same species.

The organisms identified as *S. thermophilus* were all characterised by their ability to grow at 50°C., failure to grow at 10°C., resistance to pasteurisation (63°C. for 30 minutes), rapid acid production and coagulation of milk without reduction of litmus, inability to produce acetoin from citrate or ammonia from arginine and poor tolerance to high pH, NaCl and methylene blue. The time of coagulation of plain milk at 37°C. ranged from 6 to 24 hours and the organisms were able to curdle yeast dextrose milk in 4 to 6 hours at 45 to 47°C. A firm curd with very little whey separation and a mildly acidic flavour was formed. The maximum acidity produced varied from 0·8 to 1·0 per cent lactic acid. Most of them fermented sucrose but not maltose and dextrin. Three islolates (S. 240, S. 258 and S. 252A) may be considered to be most typical of the species, although S. 252A fermented maltose. Two of them (S. 240 and S. 258) fermented dextrose very weakly; in course of time they were found to have completely lost their ability to ferment this sugar, while their capacity to produce acid from lactose and sucrose remained unaltered. The occurrence of a number of isolates in the *S. thermophilus* group, showing a similar inability to ferment dextrose, has been indicated in the preliminary classification of the organisms. This anamolous metabolic behaviour of some

Table III(*a*)

Cultural and physiological characteristics of streptococci isolated from dahi

Species	Isolates	Growth at different temperatures (°C.)			Heat resistance (63°C. for ½ hour)	Action on litmus milk †	Time of coagulation of milk 37°C. (hours)	Maximum acidity in milk (per cent lactic acid)	Volatile acidity in milk (ml. N/10 NaOH; 250 g. of culture)	Acetoin from citrate	NH3 from arginine	Alkali tolerance		NaCl tolerance			Methylene blue tolerance 0·1 per cent
		10	45	50								pH 9·2	pH 9·6	2 per cent	4 per cent	6·5 per cent	
S. thermophilus	S. 240; S. 252A; S. 258	0	+	+	+	AC	6—14	1·00	0	0	0	0	0	0	0	0	0
	S. 98; S. 197; S. 71	0	+	+	+	AC	18	0·80-1·00	0.2-7	0	0	+	0	0	0	0	0
	S. 182	0	+	+	+	AC	18	1·00	3·2	0	0	+	0	0	0	0	+
	S. 13; S. 18	0	+	±	+	AC	10—14	0·80-1·00	5·5-6·5	0	0	0	0	0	0	0	0
	S. 8 (?)	0	+	±	+	AC	24	0·91	3·5	0	0	±	0	+	+	0	+
S. lactis	S. 157	+	0	0	0	ARC	22	0·90	4·1	±	+	+	0	+	+	0	+
	S. 69; S. 154	+	0	0	0	ARC	24	0·80	3·4	0	+	+	0	+	+	0	+
	S. 10	+	0	0	0	ARC*	12	0·90	4·7	0	0	+	0	+	0	0	+
	S. 39	+	0	0	0	ARC	40	0·75	3·2	0	+	+	+	+	+	0	+
	S. 57	+	0	0	0	ARC	24	0·80	3·0	0	+	+	+	+	+	+	+
S. faecalis	S. 100; S. 1; S. 30; S. 124; S. 120	+	+	+	+	ARC	8—24	0·70-0·80	3·4-8·6	+	+	+	+	+	+	+	+
	S. 151; S. 223	+	+	+	+	ARC	3—4 days	0·70-0·80	..	0	+	+	+	+	+	+	+
	S. 4	+	+	+	+	ARC	24	0·50	3·3	0	+	+	±	+	+	0	+
	S. 59	+	+	±	+	ARC	19	0·90	3·4	+	+	+	+	+	+	+	+
	S. 168	+	+	0	+	ARC	36	0·75	0	+	+	+	0	+	+	0	±
S. liquefaciens	S. 170	+	+	±	+	RACP	18	0·90	9·4	+	+	+	+	+	+	+	+
	S. 108	+	+	±	+	RACP	18	0·55	15·3	+	+	+	+	+	+	+	+
	S. 127	+	±	+	+	ARCP	6	0·68	21·3	+	+	+	+	+	+	+	+
S. faecalis (?) (Hetero-fermentative)	S. 207; S. 269; S. 259	+	+	+	+	ARC	3—4 days	0·50-0·60	25-26	+	+	+	+	+	+	+	+
	S. 212; S. 213	+	+	+	+	ARC	3—4 days	0·50-0·60	30—35	±	+	+	+	+	+	+	+

A = Acid; R = Reduction of litmus; C = Coagulation of milk; P = Proteolysis (p = slight proteolysis) + = Positive;
± = Variable or slight; 0 = Negative. * = S. 10 gave a ropy curd.
†The order in which the letters are placed indicate the chronological sequence of change.

TABLE III (*b*)

Sugar fermentations of streptococci isolated from dahi

Species	Isolates	Arabinose	Xylose	Rhamnose	Dextrose	Mannose	Galactose	Levulose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Dextrin	Starch	Inulin	Salicin	Sorbitol	Mannitol	Glycerol
S. thermophilus	S. 240	0	0	±	0	±	0	0	+	0	+	+	+	0	0	0	0	0	0	0
	S. 252A	0	0	±	0	±	0	0	+	+	+	+	+	0	0	0	0	0	0	0
	S. 258	0	0	0	+	+	±	+	+	0	+	±	±	0	0	0	0	0	0	0
	S. 98, S. 197, S. 71	0	±	+	+	+	0	0	+	0	+	+	+	0	0	0	0	0	0	0
	S. 182	±	±	0	+	+	0	0	+	0	+	±	0	0	0	0	0	0	0	0
	S. 13	0	0	0	+	±	0	+	+	0	+	0	0	0	0	0	0	0	0	±
	S. 18	0	0	0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	0
	S. 8		±	0	+	0	0	0	+	0	+	+	0	0	±	0	0	0	0	0
S. lactis	S. 157	0	0	0	+	0	+	+	0	+	+	0	0	+	+	0	+	0	0	0
	S. 69	0	0	0	+	0	0	0	0	0	+	0	0	+	±	0	0	0	0	0
	S. 154, S. 10, S. 39	0	±	0	+	0	0	0	+	0	+	0	0	0	0	0	0	0	0	0
	S. 57	0	0	0	+	0	+	+	0	+	+	0	0	+	+	0	+	0	0	0
S. faecalis	S. 190	0	0	0	+	±	+	+	+	+	+	+	0	+	0	0	0	0	0	0
	S. 1	0	0	0	+	±	0	+	+	0	+	0	0	0	0	0	0	0	0	+
	S. 30	0	0	0	+	±	0	+	+	0	+	0	0	0	0	0	0	0	0	±
	S. 124	0	±	0	+	0	0	±	+	0	+	0	+	+	0	±	0	0	0	0
	S. 120	0	+	0	+	0	0	0	±	0	+	0	+	0	0	0	0	0	0	0
	S. 151, S. 223	+	±	0	+	+	+	+	+	+	+	+	+	+	0	0	+	0	0	+
	S. 4, S. 59, S. 168	0	0	0	+	±	0	+	+	0	+	0	0	0	0	0	0	0	0	±
S. liquefaciens	S. 170	0	±	0	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+
	S. 108	0	0	0	+	+	+	+	+	+	+	+	0	+	±	0	+	+	+	+
	S. 127	0	+	0	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+
S. faecalis(?) (Hetero-fermentative)	S. 207, S. 209, S. 253	+	+	..	+	+	+	+	+	+	+	+	..	+	±	0	+	±	+	+
	S. 212, S. 213	+	+	..	+	+	+	+	+	+	+	+	..	+	±	0	+	±	+	+

+ = Positive (acid production);
± = variable or slight;
0 = Negative.

strains of *S. thermophilus* deserves further investigation. Isolate S. 8 is an a typical strain since it was able to tolerate 4 per cent NaCl and 0·1 per cent methylene blue.

Six isolates have been identified as *S. lactis*. They were distinguished from *S. thermophilus* in their ability to grow at 10°C. and failure to grow at 45°C. or survive (63°C. for 30 minutes, production of NH_3 from arginine (except for one isolate, S. 10) and tolerance to *p*H 9·2, 4 per cent NaCl (except S. 10), and 0·1 per cent methylene blue. They were able to coagulate milk in 14 to 24 hours at 37°C. and produced a maximum acidity ranging from 0·75 to 0·90 per cent lactic acid. Isolate S. 157, which fermented maltose and dextrin but not sucrose, may be considered to be typical of the species. Two other isolates (S. 69 and S. 154) closely resembled the typical strain except for their inability to ferment maltose and the latter's ability to attack sucrose strongly. Isolate S. 10, which formed a ropy curd, showed long chains of cells and capsulated forms. It also failed to grow in 4 per cent NaCl, produce ammonia from arginine or ferment maltose and dextrin ; it thus resembled *S. cremoris* more closely than *S. lactis*. This strain is probably similar to the organisms described by Joshi and Ram Ayyar [1936] and Karnad [1939] except that it showed ropiness. On the other hand, isolate S. 57 showed some relationship to *S. faecalis* by its ability to tolerate *p*H 9·6 and 6·5 per cent NaCl.

The remaining streptococci (18 isolates) have been included in the Enterococcus group. They were able to grow at 10°C. as well as at 45°C. and, with the exception of 4 strains, showed growth even at 50°C. All of them survived pasteurisation (63°C. for 30 minutes) produced ammonia from arginine and produced acid in milk followed by reduction of litmus and coagulation of milk. With the exception of two isolates they also produced acetoin in sugar free citrate medium and did not show any inhibition at *p*H 9·6 or by 6·5 per cent NaCl and 0·1 per cent methylene blue. Ten of these have been identified as *S. faecalis*. They produced acid and coagulation of milk rapidly (8—24 hours in the case of 7 strains and 2 to 4 days in other cases), the maximum acidity produced varying from 0·50 to 0·98 per cent lactic acid. Isolates S. 190, S. 1, S. 30, S. 124 and S. 129 are typical of the species, except for their variable behaviour in fermenting maltose, arabinose raffinose, salicin, mannite and glycerol, which have been reported to be fermented by *S. faecalis* [Breed, Murray and Hitchins, 1948].

Three isolates (S. 170, S. 108 and S. 127) produced acid, reduced litmus, coagulated milk rapidly (6 to 18 hours) and caused marked proteolysis of the curd. They were, therefore, identified as *S. liquefaciens*. The maximum acidity produced by them ranged from 0·55 to 0·93 per cent lactic acid. In addition to conforming to the typical physiological reactions of the species they also produced acid from all the important sugars (sucrose maltose, trehalose, raffinose, salicin, mannite and glycerol reported to be fermented by *S. liquefaciens* [Breed, Murray and Hitchins, 1948]. Isolates S. 108 and S. 127 were found to produce appreciable amounts of volatile acidity in milk (15·3 and 21·2 ml. of N/10 NaOH per 250 g. of culture, respectively).

Isolates S. 207, S. 209, S. 253, S. 212 and S. 213, closely resembled the previous group, except for the fact that they produced acid in milk much more slowly (taking 3 to 4 days for coagulating milk) and did not bring about any proteolysis of the curd. The maximum acidity produced was 0·50 to 0·60 per cent lactic acid. As they also

produced very high amounts of volatile acidity in milk (25 to 33 ml. of N/10 NaOH per 250g. of culture) and fermented pentoses strongly, they were originally considered to be related to *S. paracitrovorous* (Leuconostoc), but their inability to produce gas from glucose, form acetoin from citrate in the absence of glucose or other source of carbohydrate [Campbell and Gunsalus, 1944] and produce amonia from arginine precluded their being placed in this category. On the other hand, they resembled the Enterococcus group more closely (particularly *S. liquefaciens*) in their ability to grow at high temperatures, resistance to pasteurisation (63°C. for 30 minutes) and their tolerance to alkaline *p*H, 6·5 per cent NaCl and 0·1 per cent methylene blue. *S. liquefaciens* has been reported to lose its proteolytic character and this organism as well as *S. faecalis* have been reported to produce volatile acidity in milk [Long and Hammer, 1936 ; Davis *et al.*, 1939]. These isolates may therefore be regarded as heterofermentative variants of *S. faecalis* or *S. liquefaciens* or they may form a distinct species in the Enterococcus group of streptococci.

Lactobacilli. The lactobacilli have been classified (Tables IV *a* and *b*) into six different species, *viz.*, *L. bulgaricus* (7 isolates), *L. lactis* (3 isolates), *L. helveticus* (2 isolates), *L. casei* (4 isolates), *L. plantarum* (3 isolates) and *L. fermenti* (3 isolates).

The isolates included under the first three species (Thermobacteria) were characterized by their ability to grow at 50°C., failure to grow at 15°C., resistance to pasteurisation (63°C. for 30 minutes) and rapid acid production and coagulation of milk in 13 to 40 hours at 37°C. Yeast dextrose milk was curdled in 6 to 10 hrs. at 45°C., and, with the exception of *L. bulgaricus* strains, all the isolates reduced litmus. The maximum acidity ranged from 1·7 to 2·2 per cent lactic acid. They formed a firm and highly acid curd with very little whey separation or volatile acid production and did not produce gas from glucose or show growth in 2 per cent lactate media. Their growth was not inhibited by 5 per cent NaCl but results regarding tolerance to 2·5 per cent NaCl and 0·1 per cent methylene blue were variable. All of them fermented dextrose and lactose vigorously. Their further differentiation was mainly based on slight morphological variations, their ability to reduce litmus, and differences in the fermentation of sugars particularly of maltose and sucrose.

The strains of *L. bulgaricus* appeared as long slender rods ($3{\cdot}0\text{-}4{\cdot}5 \times 0{\cdot}5\text{-}0{\cdot}7\mu$) usually occurring singly and occasionally in long chains or filaments, showing metachromatic granules prominently when stained with aqueous methylene blue. Agar colonies of these organisms were of the rough type and were highly filamentous. Isolates L.96, L.393 and L.409 may be considered to be typical of the species except for their tolerance to 2·5 per cent NaCl and fermentation of arabinose ; L.96 had the ability to ferment maltose slightly. One isolate (L.1) fermented arabinose and sucrose strongly and maltose slightly. These organisms appeared to be analogous to the lactobacilli associated with Yoghourt, Kefir and other fermented milks of the Mediterranean and Middle East countries although the very high values of acidity (2·5-4 per cent) reported by previous workers [Hammer, 1948] were not obtained with them. They are probably similar to the organisms described by Chatterjee [1910] under the name '*Streptothrix dadhi*' and later by Ram Ayyar [1928].

Three isolates (L.326, L.376 and L.395) have been identified as *L. lactis* mainly on account of their ability to reduce litmus and ferment sucrose, maltose, arbinose and other sugars which are not attacked by *L. bulgaricus*. Morphologically they

TABLE IV(*a*)

Cultural and physiological characteristics of lactobacilli isolated from dahi

Species	Isolates	Growth at different temperatures (°C.) 10	15	22	45	50	Heat resistance (65°C. for ½ hour)	Action on litmus milk ‡	me f coagulation of milk 37°C. hours	Maximum acidity in milk (per cent lactic acid)	Volatile acidity in milk (ml. N/10 NaoH 250g of culture	Gas from glucose	Growth in 2 per cent lactate media	NaCl tolerance 2·5 per cent	5·0 per cent	Methylene blue tolerance 0·1 per cent
L. bulgaricus	L. 96	0	0	+	+	+	+	AC	16	1·9	0	0	0	+	0	+
	L. 203, L. 400	0	0	+	+	+	+	AC	13	2·0—2·2	0	0	0	+	0	+
	L. 213, 217	0	0	+	+	+	+	AC	40	1·7	0	0	0	+	0	+
	L. 86	0	0	+	+	+	+	AC	24	1·7	0	0	0	0	0	0
	L. 1	0	0	+	+	+	+	AC	20	1·8	0	0	0	±	0	±
L. lactis	L. 326, L. 376	0	±	+	+	+	+	ACR	16	1·8 2·0	0	0	0	+	0	0
	L. 395	0	0	+	+	+	+	ACR	13	2·0	0	0	0	+	0	+
L. helveticus	L. 12	0	0	+	+	±	+	ARC	24	2·2	0	0	0	0	0	+
	L. 189	0	0	+	+	±	+	ARC	40	1·6	0	0	0	0	0	+
L. casei	L. 401, L. 401A	+	+	+	+	0	±	ARC	4 days	1·6	0	0	+	+	+	0
	L. 84, L. 134	+	+	+	+	0	±	ACr	3—5 days	1·4	0	0	+	+	0	0
L. plantarum	L. 157	+	+	+	0	0	+	AC	5—7 days	1·5	0	0	+	+	0	0
L. plantarum ? (Heterofermentative)	L. 89	+	+	+	+	0	0	ArC	2—3 days	0·8	30 ml.	0	+	+	0	0
	L. 73	+	+	+	+	0	0	ArC	5 days	1·0	20 ml.	0	+	+	0	0
L. fermenti	L. 121, L.124	0	0	+	+	0	0	A	60	0·3	High	+	+	0	0	0
	L. 414	0	0	+	0	0	0	A	60	0·3	High	+	+	0	0	0

A = Acid : C = Coagulation of milk: R = Reduction of litmus ; (r = slight reduction) ; + = Positive : ± = Variable or slight : 0 = Negative.
‡ The order in which the letters are placed indicate he chronological sequence of changes.

TABLE IV(b)

Sugar fermentations of lactobacilli isolated from dahi

Species	Isolates	Arabinoise	Xylose	Dextrose	Galactose	Levulose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Dextrin	Starch	Inulin	Salicin	Mannitol
L. bulgaricus	L.96	+	±	+	0	±	0	±	+	+	0	0	±	0	±	0
	L.213, L.217	0	±	+	+	+	0	0	+	0	0	0	0	0	0	0
	L.393, L.409	±	+	+	±	+	0	0	+	0	0	0	0	0	0	0
	L.86	0	±	+	0	+	±	0	+	+	0	0	0	0	0	0
	L.1	+	±	+	0	+	+	±	+	0	0	0	0	0	±	0
L. lactis	L.326, L.376	+	+	+	+	+	+	+	+	+	0	0	±	0	+	0
	L.395	+	+	+	±	+	+	0	+	+	±	±	0	±	+	0
L. helveticus	L.12	+	±	+	±	+	0	±	+	+	0	0	0	0	±	0
	L.189	+	0	+	0	+	0	+	+	+	0	0	0	0	0	0
L. casei	L.401, L. 401A	+	+	+	+	+	+	+	+	+	0	+	±	0	+	+
	L.84, L.134	+	+	+	+	+	+	+	+	+	0	0	0	0	+	0
L. plantarum	L.157	±	+	+	+	+	+	+	+	+	+	±	±	+	+	+
L. plantarum (Heterofermentative)	L.89	+	+	+	+	+	+	+	+	+	0	0	±	±	0	0
	L.73	+	+	+	+	+	+	+	+	+	0	0	±	0	+	0
L. fermenti	L.121, L.124	0	0	+	0	0	0	0	+	0	0	0	0	0	0	0
	L.414	0	0	+	0	0	0	0	+	0	0	0	0	0	0	0

+ = Positive (Acid production) ; ± = Doubtful or slight 0 = Negative.

appeared to be slightly different from *L. bulgaricus* as they showed many curved and coiled forms and occasionally contained more numerous granules in the cells. In all other respects these organisms closely resembled the *L. bulgaricus* strains.

Isolates L.12 and L.189, which have been identified as *L. helveticus* differed from the above two species in appearing as shorter and thinner cells arranged in pairs of palisade formation almost resembling the cells of *L. casei*. The colonies formed were generally of the 'smooth' type. The organisms were inhibited by 2·5 per cent NaCl but not by 0·1 per cent methylene blue. Arabinose and maltose were fermented while sucrose, raffinose and salicin were not attacked.

The organisms included under *L. casei* and *L. plantarum* species (Streptobacteria) were able to grow at 10°C. and with the exception of one isolate (L.157), at 45°C. None of them showed growth at 50°C. Resistance to pasteurisation (63°C. for 30 min.) was, however, variable. They produced acid in milk more slowly than the Thermobacteria and took 3 to 4 days or longer to coagulate milk at 37°C. Maximum acidity produced was 1·4 to 1·6 per cent lactic acid in the case of *L. casei* strains and less than one per cent in others. They did not show any gas formation from glucose and with the exception of two isolates (L.89 and L.73) they did not produce any volatile acidity in milk. They were able to grow in two per cent lactate and tolerate 2·5 per cent concentration of NaCl. All of them were inhibited by 0·1 per cent methylene blue. In morphology, they were clearly distinguished from *L. bulgaricus* and related strains by their appearance as very short and thin rods (1·5-2·5×0·2μ) occurring in pairs, chains, palisade formation and occasionally in long filaments. Granules were not prominent. In the case of L.157, two types of cells—one short and thin and the other larger in size with rounded ends—were seen. Capsulated cells were shown by the heterofermentative strains (L.89 and L.73) in India ink preparations of the smears. The colonies formed by all these organisms were of the 'smooth' type. All the isolates fermented arabinose, xylose, dextrose, galactose, sucrose, maltose, lactose, trehalose and generally salicin and mannitol.

Isolates L.401, L.401 *a*, L.84 and L.134, appeared to be typical strains of *L. casei* from their morphology, reduction of litmus and rate of acid production, maximum acidity produced (1·4-1·6 per cent lactic acid), sugar fermentations and their ability to tolerate 5 per cent NaCl (except for isolates L.84 and L.134). Isolate L.157 has been identified as *L. plantarum* on account of some differences shown by the organism in respect of morphology, rate of acid production in milk (taking 5 to 7 days to coagulate milk at 37°C.), inability to reduce litmus, and low maximum acidity produced (0·5 per cent lactic acid).

Isolates L.89 and L.73 closely resembled the *L. plantarum* strain except for their ability to produce high volatile acidity in milk (20-30 ml. of N/10 NaOH per 250 g. of culture). The curd formed was weak in texture but was associated with a strong diacetyl flavour. These organisms have been considered to be heterofermentative variants of *L. plantarum* and not included under Betabacteria in view of their comparatively rapid rate of acid production in milk, failure to produce gas from glucose and marked fermentation of arabinose. Similar strains of *L. plantarum* do not appear to have been reported in fermented milks, although Sherwood [1939] has referred to their occurrence in cheese and suggested the possibility of their being intermediate forms between *L. casei* and *L. brevis*.

Three isolates (L.121, L.124 and L.414) were found to possess the characteristics of Betabacteria. They grew very slowly in milk producing only acid, and milk was not coagulated even when incubated for over one month at 30°C. (maximum acidity being 0·3 per cent lactic acid). Addition of yeast extract stimulated acid production and a weak curd was formed after 10 to 15 days. The organisms produced gas from glucose and high amounts of volatile acidity in milk. They grew in two per cent lactate medium but were inhibited by 2·5 per cent NaCl and 0·1 per cent methylene blue. In morphology they resembled *L. casei* strains except that slightly longer cells were also encountered. The colonies formed were of the smooth type. Dextrose and lactose were the only two sugars which were fermented to any appreciable extent. The organisms were able to grow at 45°C. (except for isolate L.414), but not at 15°C. In view of this property, as well as their inability to ferment arabinose, the isolates have been identified as *L. fermenti*.

Summary

Studies on the isolation, characterization and classification of 290 streptococci and 430 lactobacilli, isolated from over 500 samples of dahi collected from different parts of India, are reported.

On the basis of certain important characteristics, the isolates were broadly classified into different groups. The streptococci were divided into 4 groups, *viz.*, *S. thermophilus* (140 isolates), *S. lactis* (28 isolates) *S. faecalis* (107 isolates) and *S. dextranicum* (15 isolates). The Lactobacilli, were classified into 3 groups, *viz.*, *L. bulgaricus* (310 isolates), *L. casei* (88 isolates) and *L. brevis* (32 isolates).

Representative isolates selected from each of the above groups were studied in detail for their species identification. The species of streptococci identified included *S. thermophilus*, *S. lactis*, *S. faecalis*, and *S. liquifaciens* while amongst the lactobacilli, *L. bulgaricus* *L. lactis*, *L. helveticus*, *L. casei*, *L. plantarum* and *L. fermenti* were encountered. Some heterofermentative streptococci and lactobacilli were identified as variants of *S. faecalis* and *L. plantarum* respectively.

Amongst the streptococci isolated from *dahi*, *S. thermophilus* and *S. faecalis* were encountered most frequently while *L. bulgaricus* was the most commonly occurring lactobacillus in this fermented milk.

The heterofermentative streptococci and lactobacilli were almost invariably associated with *dahi* of good aroma and other desirable qualities, obtained from centres in Uttar Pradesh, Bihar and West Bengal.

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REDUCTION IN MILK YIELD IN INDIAN DAIRY CATTLE BY ARTHROPOD INVASION AND ITS CONTROL

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It is well known that ectoparasites when present in large numbers cause annoyance and irritation to animals. Sometimes their intensity is so great that the animals are worried day and night. The annoyance caused by them is manifested in various ways. At times the cows are so frightened by the attack of *Lyperosia exigua* that while grazing they huddle together so as to give one another as much protection as possible. They scatter themselves to prevent the attack from sand-flies, the animals keep constantly walking and thus keeping their condition down. Buffalo flies cause sores about the eyes, dewlap and body of the animals and thus cause constant worry to them. Constant biting of ticks makes them irritated and weak due to loss of blood.

The use of insecticidal sprays to save cows from the attack of the biting flies is now considered one of the effective methods in all progressive countries of the world. The use of the fly trap can be of value only on a small scale as in a dairy farm where cattle are quiet and can easily be trained to go through. Moreover, fly traps cannot be of any use in ridding the animals of parasites like lice, ticks, etc.

The factors which determine the production of milk are so numerous that a large number of controlled observations are considered necessary to find out an exact relationship between the invasion of ectoparasites and the production of milk. Various opinions have already been expressed on the effect of ectoparasites on milk production and on the fattening of cattle.

Freeborn *et al.* [1925] appear to be the first whose attention was drawn to this problem and they conducted a large series of experiments on scientific lines to evaluate varying popular belief about it. From their series of experiments it was shown that 9·26 per cent of the loss in milk flow was caused by stable flies (*Stomoxys calcitrans*), 3·33 per cent by house flies (*Musca domestica*), 1·4 per cent by horn flies (*Siphona irritans*) and 4·11 per cent by the use of certain repellent sprays. Later on Bishopp [1930] recorded that the farmers estimated 40 to 60 per cent reduction in milk yield as due to stable fly invasion. It is not quite clear from his report if this estimate was based on scientific data. Sen [1939] carried out experiments on five Sahiwal cows and concluded that an appreciable increase in the yield of milk could be effected by controlling *Lyperosia exigua and Musca crassirostris* in India. Belschner [1945] recorded a drop of 15 to 50 per cent in milk yield and from a few months to a few years delay in fattening of cattle due to an attack of buffalo flies, *Lyperosia exigua* alone, though he felt that the statement needed confirmation by careful planned experiments. Later on, Bruce and Decker

[1947] in their well planned experiments showed that there was a high degree of inverse correlation between changes in milk production and fly abundance. Thus there is no doubt that fly worry does result in a drop in milk production and some delay in fattening.

Considering the seriousness of shortage in milk production in India and the very limited number of experiments conducted on the subject, this problem was taken up by the Indian Veterinary Research Institute with a view to finally assess the amount of loss in milk production in this country due to arthropod invasion and to find out a suitable control measure to combat the arthropods at a very low cost. The results so far obtained from the present series of experiments are recorded below. Further work on the subject is in progress.

MATERIAL AND METHODS

The insecticidal sprays used are as follows :

A	High speed Diesel oil	92 parts
	Pyrocide 20	5 parts
	Pine oil	3 parts
B	Ext. Pyrethrum Liquid (2 per cent)	5 parts
	Kerosine oil	95 parts
C	DDT	1 gm.
	Kerosine oil	15 c.c.
	Water	1000 c.c.
D	Ext. Pyrethrum Liquid (2 per cent)	5 c.c.
	DDT	0·5 gm.
	Kerosine oil	95 c.c.

The animals were mostly of Hariana breed from the farm and had nearly the same lactation period. The sprays were applied all over the body excepting eyes and udder by a mish sprayer every day at six O'clock in the morning. The parts which received more attention were the neck and the wither. Approximately 60 c.c. of the mixture was used per animal per day. The operation was continued daily for one month and separate controls were kept for each series of experiments. Five such series of experiments were conducted, and the milk records not only for the period during which the spraying operations were carried out but also for one month both preceding and following the operation were kept. The five series of experiments were conducted during the different seasons of the year, *viz.*, the hot, the monsoon, the post-monsoon and the cold months. January and February were taken as cold months, April and May as hot months, August and September as monsoon period and October and November as post-monsoon months of Izatnagar, Bareilly. Incidentally it may be mentioned that the area has extremes of climate, the temperature varying from a maximum of 115° F. in hot months to a minimum of 41° F. in cold months. The rainfall during monsoon months is usually 42·15 inches.

The ectoparasites commonly recorded in the area on cattle are: (1) *Musca* (mostly *nebulo* Wied.) (2) *Musca crassirostris* Stein (3) *Lyperosia exigua* de Meij. (4) *Stomoxys calcitrans* (Linn.) (5) *Hippobosca maculata* Leach (6) *Hyalomma aegyptium* Linn. and (7) *Boophilus australis* Fuller. Nos. 6 and 7 were found more or less throughout the year though in the months of January and February they w more prevalent. Nos. 1 to 4 were found frequently attacking the animals.

In order to evaluate the increase or decrease in milk yield the following procedure was adopted. In each group the milk record was reduced to the number of pounds per cow per month for the periods during which spraying operation was conducted as well as for one month both preceding and following. In Tables I to V the milk yield per cow per month preceding the application of spray was taken as the base and in Tables VI to IX the figures for the period when the spraying was done was taken as the base. Over these bases the percentage of milk yield was calculated and was compared with the controls to find out the apparent increase or decrease in milk yield.

Experiment 1

Twenty animals used for this experiment were divided into four groups, three groups receiving one of the sprays A, B and C and Group E served as control. The spraying operation was performed in hot weather for one month during April to May 1948 when the density of ectoparasites was at a low level. The result of the experiment is presented on Table I. It may be seen from the Table that there was an apparent increase of 4·7 to 10·3 per cent in milk yield over the controls.

TABLE I

Effect of various insecticides on milk yield during hot season.

Group receiving the spray	Number of animals in the group	lb. per cow per month for a period		Per cent of base	Per cent apparent increase over control
		11-3-48—9-4-48	10-4-48—9-5-48		
A	5	517	482	93·2	+4·7
B	5	371·4	351	94·5	+6·0
C	5	337·4	333·6	98·8	+10·3
E (control)	5	283·6	251·2	88·5	..

Experiment 2

Twenty five animals were used for this experiment. They were divided into five groups, out of which four groups received the different sprays A, B, C and D and one group E remained as control. The spraying was done for a month during August-September 1948 when *Lyperosia exigua* (de Meij.) and *Stomoxys calcitrans* were found in fair numbers. *Musca.* (mostly *nebulo* Wied.) was present in very

large numbers. The result of this experiment is given below in Table II. Here also there was an apparent increase of 2·2 to 11·0 per cent in milk yield over the controls.

TABLE II

Effect of various insecticides on milk yield during monsoon season

Group receiving the spray	Number of animals in the group	lb. per cow per month for the period		Per cent of base	Per cent apparent increase over control
		19-7-48—18-8-48	19-8-48—18-9-48		
A	5	240·6	236·5	98·3	+11·0
B	5	188·0	184·8	92·3	+5·0
C	5	285·0	258·4	90·7	+3·4
D	5	270·3	241·8	89·5	+2·2
E (control)	5	363·2	317·0	87·3	..

Experiment 3

Sprays A, B, C and D were tested on four groups, each group consisting of four animals. Group E consisting of four animals served as control. The spraying operation was conducted for one month during October-November 1948. The following ectoparasites were prevalent during the period : (1) *Hippobosca maculata* Leach (2) *Lyperosia exigua* (de Meij.) (3) *Stomoxys calcitrans* (Linn.) and (4) a large number of *Musca* (mostly *nebulo* Wied.). The result of the experiment is shown in Table III. It may be noticed from the table that here also there is an apparent increase of milk yield from 4·2 to 21·7 per cent over controls in groups B, C and D, though there was a little decrease of 2·7 per cent in group A. It is rather difficult to explain this decrease.

TABLE III

Effect of various insecticides on milk yield during past monsoon season.

Group receiving the spray	Number of animals in the group	lb. per cow per month for the period		Per cent of base	Per cent apparent increase over conrol
		9-9-48—8-10-48	9-10-48—8-11-48		
A	4	247·5	214·5	86·7	—2·7
B	4	336·0	314·5	93·6	+4·2
C	4	414·0	442·2	100·7	+11·3
D	4	400·5	465·0	111·1	+21·7
E (control)	4	449·0	401·5	89·4	..

Experiment 4

Fifteen animals used for this experiment were divided into five groups of three animals each, the first four groups receiving sprays A, B, C and D and the last group E remaining as control. The spraying was done for one month during January-February 1949. *Lyperosia exigua* (de Meij.), *Stomoxys calcitrans* (Linn.) and *Musca* (mostly *nebulo* Wied.) were present. The result of this experiment is tabulated on Table IV from which it may be seen that there was an apparent increase of 3·2 to 9·4 per cent in milk yield over controls.

TABLE IV

Effect of various insecticides on milk yield during cold season

Group receiving the spray	Number of animals in the group	lb. per cow per month for the period		Per cent of base	Per cent apparent increase over control
		20-12-48—19-1-49	20-1-49—19-2-49		
A	3	292·0	248·5	85·1	+3·2
B	3	256·3	238·0	92·9	+11·0
C	3	462·5	417·5	90·3	+8·4
D	3	255·0	233·0	91·3	+9·4
E (control)	3	429·3	351·7	81·9	..

Experiment 5

As in the previous series, 15 animals were used for this experiment. They were divided into five groups of three animals each, four groups receiving the four different sprays A, B, C and D and one group E remaining as control. The spraying operations were conducted for a month during May 1948. *Hippobosca maculata* Leach and *Musca* (mostly *nebulo* Wied.) were present. The result of the experiment is given on Table V. It may be seen from the table that in all groups of sprayed animals there was an apparent increase in the milk yield varying from 10·1 to 12·4 per cent over controls.

TABLE V

Effect of various insecticides on milk yield during hot season

Group receiving the spray	Number of animals in the group	lb. per cow per month for the period		Per cent of base	Per cent of apparent increase over control
		1-4-49—30-4-49	1-5-49—30-5-49		
A	3	365·3	345·7	94·6	+12·4
B	3	245·7	228·7	93·2	+11·0
C	3	325·7	300·5	92·3	+10·1
D	3	208·7	197·7	92·8	+10·6
E (control)	3	464·0	381·3	82·2	..

The record of milk yield for one month of the cows under the various experiments following the application of the spray is presented on Tables VI to IX.

It will be noticed that the improvement in milk yield has been maintained in all the series of experiments even though spraying had actually been stopped by them. This can only be attributed to the continued effect which the spray had in keeping the ectoparasites in check and the consequent improvement in the milk production.

The data of milk yield for the period after spraying for Experiment 4 has not been tabulated as the milk record figures were not available, some of the animals being sent outside for other purposes.

TABLE VI

Effect on milk yield following application of various insecticides during hot season

Group receiving the spray	Number of animals in the group	lb. per cow per month for the period		Per cent of base	Per cent of apparent increase over control
		10-4-48—9-5-48	10-5-48—8-6-48		
A	5	482·0	423·0	87·7	+3·4
B	5	351·0	302·6	86·2	+1·9
C	5	333·6	275·8	82·7	−1·6
E (control)	5	251·2	211·8	84·3	

TABLE VII

Effect on milk yield following application of various insecticides during monsoon season

Group receiving the spray	Number of animals in the group	lb. per cow per month for the period		Per cent of base	Per cent of apparent increase over control
		19-8-48—18-9-48	19-9-48—18-10-48		
A	5	236·5	220·6	93·3	+1·1
B	5	184·8	166·4	90·0	−2·2
C	5	258·4	259·6	100·4	+8·2
D	5	241·8	203·3	84·0	−8·2
E (control)	5	317·0	292·2	92·2	..

TABLE VIII

Effect on milk yield following application of various insecticides during past monsoon season.

r u receiving the spray	Number of animals in the group	lb. per cow per month for the period		Per cent of base	Per cent of apparent increase over control
		9-10-48—8-11-48	9-11-48—8-12-48		
A	4	214·5	183·0	85·3	+12·4
B	4	314·5	265·5	81·2	+8·3
C	4	442·2	371·0	83·9	+11·0
D	4	465·0	442·5	95·2	+22·3
E (Control)	4	401·5	292·5	72·9	..

TABLE IX

Effect on milk yield following application of various insecticides during hot season

Group receiving the spray	Number of animals in group	lb. per cow per month for the period		Per cent of base	Per cent of apparent increase over control
		1-5-49—30-5-49	31-5-49—29-6-49		
A	3	345·7	298·0	86·3	+11·6
B	3	228·7	189·3	82·7	+8·0
C	3	300·5	248·0	82·5	+7·8
D	3	193·7	170·0	87·9	+13·2
E (control)	3	381·3	285·0	74·7	..

The detailed result of the comparative efficacy of the four different sprays used in our series of experiments may be seen from Table X.

The spray D which is a mixture of pyrethrum and DDT is decidedly superior to the other ones so far as increse in milk yield is concerned. With regard to the other sprays, *viz.*, A, B and C there is a slight variation in the results during the period of spraying and its after effects, whereas spray D has given consistently good results.

TABLE X

Per cent apparent increase or decrease in milk yield over control in all experiments with the group receiving spray

A		B		C		D		Season
During spray	After spray	During spray	After spray	During spray	After spray	During spray	After spray	
+4·7	+3·4	+6·0	+1·9	+10·3	—1·6	+2·2	—8·2	Hot
+11·0	+1·1	+5·0	—2·2	+3·4	+8·2	+21·7	+22·3	Monsoon
—2·7	+12·4	+4·2	+8·3	+11·3	+11·0	+9·4	+13·2	Post-Monsoon
+3·2	+11·6	+11·0	+8·0	+8·4	+7·8	+10·6	..	Cold
+12·4	..	+11·0	..	+10·0	..	..	..	Hot
Average								
+5·7	+7·1	+7·4	+4·0	+8·7	+6·1	+10·9	+9·1	

From the above table it is also seen that during the period of spray the increase in milk yield varied between 4·7 and 12·4 per cent in the series of experiments conducted during the hot season; between 3·4 and 21·7 per cent during the monsoon period; between —2·7 and 11·3 per cent during the post-monsoon period and between 3·2 and 11·0 per cent during the cold season.

It is generally noticed that the incidence of arthropods is considerably reduced during the hot months and the animals are given frequent baths. During the monsoon, the insecticidal substances are generally washed out when the animals are out in the rain. During the post-monsoon months, the incidence of arthropods is decidedly increased and during the cold months the animals are not inclined to have frequent baths and as such the insecticides applied are not likely to be washed out.

DISCUSSION

It will be clear from the foregoing observations that in our series of experiments a definite increase in the yield of milk is noticeable in the sprayed animals as compared to the unsprayed controls. In Australia the decrease in milk yield due to one species of biting fly, *Lyperosia exigua*, has been reported to be as much as fifty per cent [Belschner *loc. cit.*]. Such a high percentage has not been recorded for any other country so far. The Australian report shows that only one species may be responsible for reduction by fifty per cent whereas in India we have more than half a dozen important species which infest our dairy cattle. Yet our maximum record of only twenty-two per cent reduction may be explained by the fact that in Australia the density of this single species is very high. Moreover, our observation was made in a government dairy herd where the general hygienic condition is decidedly better than that of a private farm. If this series of experiments are conducted in a private farm the percentage may be higher. But the maximum percentage of twenty-two is very significant in our country which is much below self sufficiency level in milk supply, whereas in other countries there is surplus. It will be of great

help to our country to become self sufficient in milk supply if this large quantity of milk which is lost annually due to arthropod invasion could be restored by using proper control measures. Much more work will have to be done to find out the most optimum spray fluid, the per capita cost of which could be kept at a low level so as to make it economically feasible for the average farmer or villager to use it on his cattle without any risk.

Summary

Five series of experiments were conducted in different months of 1948-49 on 95 dairy cows with four insecticidal sprays in order to ascertain the effect of ectoparasite control on milk yield.

The experimental animals of each series were divided into four groups and each group was treated with a different insecticidal mixture for a period of one month. About 60 c.c. of the mixture was sprayed daily on each animal and the cost of this quantity was calculated to be slightly less than an anna.

The milk record was maintained for the period during which the spraying operation was carried out and for one month both preceding and following the operation. Equal number of control animals were also kept for each series of experiment.

The use of the sprays not only reduced the ectoparasites but was also found to result in an appreciable increase in yield of milk.

Of the four different sprays, A, B, C and D the spray D which is a mixture of pyrethrum and DDT was found to be more effective and has given consistently good results.

The effect of the various insecticides was studied in relation to the various seasons also.

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CLIMATE AND ANIMAL HEALTH

I.—SEASONAL VARIATIONS IN THE PULSE RATE, RESPIRATION RATE, BODY TEMPERATURE, BODY WEIGHT AND HAEMOGLOBIN IN NORMAL INDIAN CATTLE

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(With one text figure)

PHILLIPS [1950] pointed out that livestock improvement work is fundamental to the improvement of agriculture in any country, both to ensure proper balance in the diets of the people and to ensure adequate humus for the soil. In order to obtain animals which will give optimum returns of milk, eggs, meat and wool or work under different climatic conditions, it is necessary to concentrate not only on the inheritance of high rates of production but also the ability to maintain these high rates under conditions prevailing in different areas. Rhoad [1941], Brody [1945, 1948], Phillips [1948, 1950], Lee [1949] and Mullick [1950] have also stressed the importance of climatic factors for the improvement of livestock. Lee and Phillips [1949] further emphasised that among such conditions which tend to limit the development of innate potentialities, the most important are high temperature, parasitic infection, nutritional deficiencies or irregularities and limited facilities for management.

Although considerable work has been done on the genetical and nutritional aspects of this problem, the studies on the seasonal variation and physiological reaction have been few and uncorrelated.

A systematic investigation was, therefore, started to study the health and general condition of animals as affected by environmental conditions, *viz.*, air temperature and moisture and to find out a suitable physiological index for heat tolerance which would be of help in distributing animals in different regions of the Indian Union according to environmental conditions.

Experimental

Six adult steers were selected for these studies. The animals were fed the same maintenance ration for the whole period of observation. Pulse rate was counted from the coccygeal artery and respiration rate from the flank movements. The animals were fed after recording body weight and rectal temperature. Drywet bulbs and maximum-minimum thermometer readings were obtained from the standard thermometers between 2 to 3 p.m. on three consecutive days in each month. Blood hæmoglobin was estimated by the acid hematin method of Newcomer [1922], by ear vein puncture.

TABLE I

Observation on pulse rate, respiration rate, body temperature, body weight and haemoglobin of the steers

Months	Air temperature °F.	Relative humidity per cent	Pulse rate (2) per minute	Respiration (2) rate per minute	Body (2) temperature °F.	Body weight (2) pounds	Hæmoglobin (1) gm./100 ml.
May	90	26	58	29	101·3	367	10·4
June	99	23	59	35	101·3	365	10·4
July	84	81	45	19	100·3	359	9·1
August	83	85	46	14	101·2	370	8·4
September	84	82	50	20	101·2	380	8·9
October	85	66	58	23	100·9	379	8·5
November	76	43	56	16	101·2	393	9·7
December	68	53	53	12	100·8	397	11·0
January	67	47	50	13	100·9	400	11·6
February	66	58	50	10	100·3	393	12·0
March	75	47	47	13	101·0	387	10·3
April	86	44	49	23	101·3	402	10·3
May	91	47	51	29	101·6	352	9·7
Range	66-99	23-85	45-59	10-35	100·3-101·6	352-402	8·4-12·0
Mean	81·1	54·0	51·6	19·7	101·0	380·0	10·0
Standard deviation	±10·1	±19·9	±4·8	±7·7	±0·41	±17·0	±0·62

(1) Hæmoglobin values are averages of 6 animals.
(2) The rest are averages of 3 consecutive days.

TABLE II

Results of analysis of variance for various characters

Sources of variations	Degrees of freedom	Characters								Degrees of Freedom		
		Pulse rate minute		Respiration rate minute		Body temperature °F		Body weight pounds			Hæmoglobin gm. 100 ml. blood	
		s.s.	m.s.	s.s.	m.s.	s.s.	m.s.	s.s.	m.s.		m.s.	s.s.
Between months	12	4622·7265	385·2272**	14561·6667	1213·4722**	3036·8889	253·0741	60194·0174	5016·1681**	12	91·1780	7·5981*
Between animals.	5	2648·3590	529·6718**	1316·0513	263·2103	987·9457	197·5897**	68404·2564	13680·8513**	5	42·2514	8·4502**
Interaction months and animals	60	2078·1966	34·6366**	1480·9487	24·6825**	801·5291	13·3588	19127·5213	318·7920**	..	..	..
Error	156	1577·3333	10·1111	691·3333	4·4316	3021·3333	19·3675	6890·6667	44·1709	60	40·2030	0·6700
Total	233	10926·6154	..	18050·0000	..	7848·0000	..	154310·4615	..	77	173·6330	..

** Highly significant value of F. ratio is obtained.

TABLE III

Partial correlations of different physiological reactions

Variables	RH held constant	Variables	AT held constant
AT× P	0·312	RH× P	—0·494*
× R	0·934*	× R	—0·681*
× BT	0·500*	× BT	—0·238
× BW	—0·350*	× BW	—0·322
× Hb	—0·724*	× Hb	—0·268

* Significant at 5 per cent level. AT—air temperature, RH—relative humidity, P—Pulse, R—respiration, BT—body temperature, BW—body weight, Hb—hæmoglobin

TABLE IV

Multiple regression equations

Estimated values	Equations
Pulse rate	0·321T—0·117H+32·31
Respiration rate	0·750T—0·096H—35·57
Body temparature °F	0·021T—0·005H+99·57
Body weight lbs.	—0·121T—0·226H+402·00
Hæmoglobin, gm.	—0·022T—0·009H+12·26

T—air temperature, in F. H—relative humidity in per cent

RESULTS

The results are given in Table I. The statistical analysis, analysis of variance, partial correlation coefficients and multiple regression equations, [Snedecor, 1946] are shown in Tables II, III and IV.

Climate. It was observed from Table I that the maximum and minimum mean temperatures were 99°F. and 66° F. The highest temperature in June was generally accompanied by the lowest humidity value. At this time 'loo' (dry hot wind), blows with great velocity and wide variations are observed in the daily maximum and minimum temperatures (106 and 85). The highest relative humidity was 85 per cent in August and lowest was 23 per cent in June, with an average of 54 per cent. There was a sudden increase in the humidity from 23 per cent in June to 81 per cent in July after the outbreak of monsoon.

Pulse. The same table shows that the maximum and minimum pulse rates were 59 in June and 45 in July, with an annual average of 52 beats per minute. With the onset of the rainy season, the pulse rate declined by 15 and it took some time for the animals to get used to the change in weather when the pulse rate gradually returned to normal. The variations in the rate between months and animals were highly significant according to analysis of variance (Table II). Using partial correlation coefficient whereby, the effect of one variable could be held constant, the correlation between air temperature and pulse rate became not significant whereas when the air temperature was held constant, the relationship between relative humidity and pulse rate was highly significant with a reverse order (Table III).

A comparison of the multiple regression co-efficient in the regression equation for the pulse rate in Table IV, appears to suggest that one degree rise in air temperature is about three times as important as one per cent decrease in relative humidity so far as their influence on pulse rate is concerned.

Respiration

Table I shows that the maximum and minimum respiration rates were 35 in June and 10 in February, with an average of 19·6 per minute. With the onset of the monsoon there was a decline in rate from 35 to 10 per minute and it remained practically the same throughout this period. The analysis of variance showed that the changes due to months and between the animals were highly significant.

The partial correlation coefficient showed that both the values due to air temperature and relative humidity were highly significant and the latter had an opposite effect on the respiration rate.

An examination of the regression coefficient shows that every degree increase in air temperature was responsible for 8 times as much increase in the rate of respiration as was caused by one per cent increase in relative humidity.

Body temperature

The maximum and minimum body temperatures were 101·6° F. in May and 100·3° F. in February with an average of 101·0° F.

There was no variation between the months but within the animals the changes were significant by the above method.

The value between air and body temperature was significant when humidity was held constant but when air temperature was kept constant it was not significant though it had an opposite effect (Table II).

Table III shows that each degree rise in air temperature is about four times as efficient as one per cent decrease in relative humidity in the change in body temperature. Humidity seems to lower the body temperature.

Body weight

The maximum and minimum body weights (Table I) were 402 in April and 359 in July with an average of 380 pounds. There were significant variations both between the months and the animals.

The correlation coefficients showed that a significant relation exists between the atmospheric temperature and live weight. The same was true in the multiple regression equation (Table IV) where humidity was the dominant factor for reducing the body weight.

Haemoglobin

The maximum and minimum values were 12·0 in February and 8·4 in August with an average of 10·0 gm. hæmoglobin per 100 ml. of blood (Table I).

The variations due to months and animals were highly significant.

The correlation between air temperature and hæmoglobin was highly significant and both the temperature and the moisture exercised an influence in lowering the values. The same was true also in multiple regression equation but the air temperature had more influence than moisture.

Discussion

The above results indicate that air temperature appears to be a major factor for influencing variations in pulse rate, respiration rate, body temperature, body weight and hæmoglobin content in animals (Tables III and IV). From Table I, it appears that relative humidity below 80 per cent plays a minor role in influencing variations in the physiological reactions. These observations are in agreement with those of Rhoad [1936], Regan and Gaalaas [1945], Seath and Miller [1946], Richardson [1938], and Reik and Lee [1948]. An examination of Fig. 1 reveals that changes in the air temperature and relative humidity were closely related to variations in the constituents recorded. The trends indicate that a clear correlation exists between air temperature and the physiological reactions. Pulse rate and body temperature changes show that only a small relationship existed with air temperature.

It is noted that the most variable physiological reaction with the change in the air temperature is the respiration rate. In comparatively less sweating animals, like cattle, one of the physical mechanisms is to compensate for their inability to sweat by the acceleration of the respiration rate. Thus this rate is first affected due to the changes in the environmental conditions long before the changes in other physiological mechanisms occur. The present investigation has shown that the change in the respiratory rate reflects even the minute changes in the air temperature. It appears, therefore, that respiration rate is the most sensitive index of the animal's feeling of discomfort.

In an attempt to express the rather complex physiological value in a single figure, Rhoad [1944] has devised a formula for calculating heat tolerance in cattle. This formula better known as 'Iberia formula' has been widely used for assaying the heat tolerance of animals in other countries. After critical examination, it is found that there are some limitations in applying it to the animals in the tropics. The atmospheric temperature in the shed (85-95° F. or higher) may have to be worked out in the tropics according to the adaptability of the animals under existing conditions. This can be seen by comparing the results of the studies undertaken by Seath and Miller [1947] and the present authors, as both have been made under

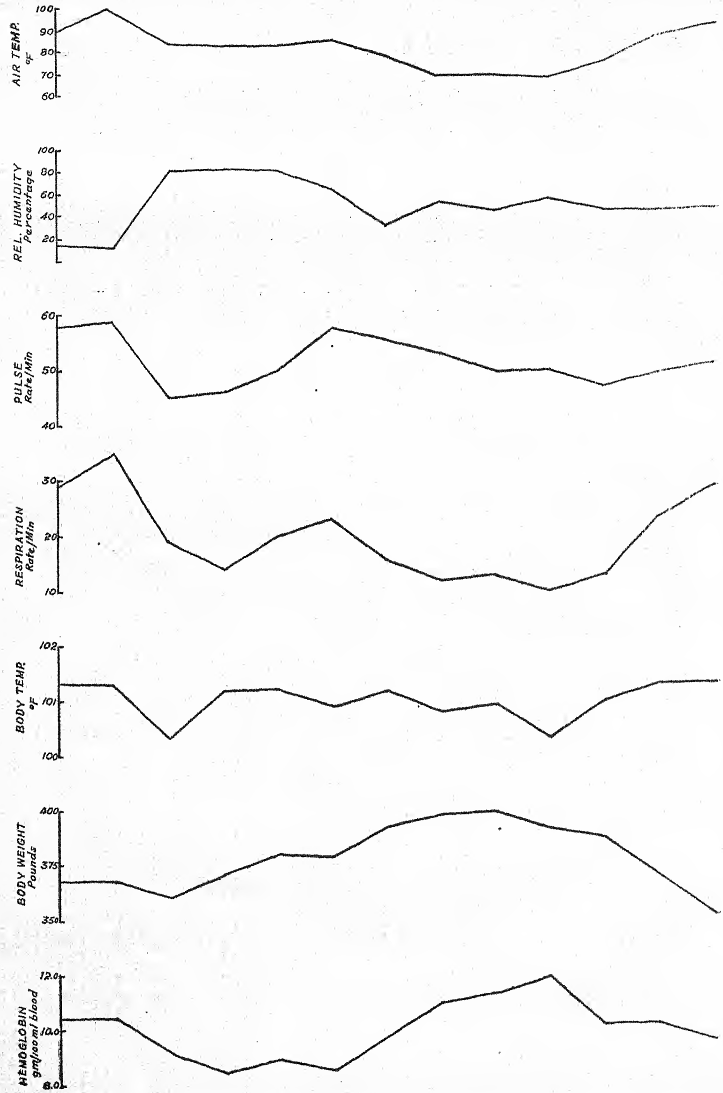

Fig. 1. Air temperature, relative humidity, pulse rate, body temperature, body weight, and hæmoglobin for variations of the animals

approximately identical conditions of air temperature and humidity. The constituents recorded, however, differed considerably and these differences could be explained by differences in the adaptability of the animals used in the two countries.

It may be mentioned that according to Iberia formula it is presumed that the rectal temperature is 101·0 F. According to our experience the rectal temperature of different breeds of cattle as also the different species of animals differs. This variation is likely to give variable results in calculating the heat tolerance index of the animals. It may be added that when the rectal temperature of animals has passed the upper critical temperature, the physiological mechanism appeared to have lost its capacity for maintaining it within the normal range of variation; thus resulting in increased temperature.

We have found that the change in respiration rate under different climatic conditions is an advantage if considered side by side with the Iberia formula for assessing heat tolerance test in cattle. The following procedure is adopted:

The pulse and respiration rates and body temperature are recorded in animals under different atmospheric conditions in the experimental stall. The animals which show the least variations in the maximum and minimum respiration rates under different climatic conditions may be considered as the best group to withstand the climatic stress of the place. Similar studies, at present under investigation, on different breeds of cattle and of different species of animals show that the respiration rate is most susceptible to variations in air temperature and moisture.

The details of this procedure in its application to cattle and buffaloes will be published in a separate article.

The fluctuations in the body weight of the animals may be directly related to the difference in the intake of feed under different environmental conditions. Recently, Ragsdale, Brody, Thomson and Worstell [1948] showed that 'physiological wisdom dictates the reduction of feed consumption with increasing temperature so as to reduce the heat production associated with feeding, which the animal cannot dissipate, and this is what happens'. Brobeck [1948] stated 'food intake appears to be controlled as if it were a mechanism of temperature regulation'.

The figures in Table I show that the hæmoglobin is minimum during the monsoon months. Present observations suggest that Indian cattle have greater ability to sweat than European animals and so can dissipate body heat by evaporative cooling through the skin by decreasing the formed elements in blood during the moist season. Kelley [1932] found that one-half zebu and one-fourth zebu cattle had 9·33 and 5·25 sweat-glands in the same area of the skin.

Summary

In this article, which is the first of this series on climate and animal health, observations on pulse rate, respiration rate, body temperature, body weight and hæmoglobin were made at monthly intervals on six Kumauni steers. Records of mean temperature and relative humidity were also maintained.

Changes in the air temperature appeared to be the major cause for affecting variations in these physiological reactions of animals. Higher values for pulse rate, respiration rate and body temperature and lower values for body weight and

hæmoglobin were recorded with rise in air temperature. Change in the respiration rate was most conspicuous.

It appeared that a relative humidity above 80 per cent had some influence in causing the variations of the reactions.

Analysis of variance, partial correlation coefficient and multiple regression equations were used for statistical analysis of the data.

It appears that the respiration rate is one of the indices for assaying heat tolerance of animals.

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ABSTRACTS

Immunity in cattle vaccinated with *Brucella abortus* strain 19 by the subcutaneous and intracaudal routes during calfhood. BUDDLE, M. B. (1948). *Aust. Vet. J.* *24*, 262-271

IN this paper a comparison has been made of the immunity of groups of heifers vaccinated during calfhood with *Brucella abortus* strain 19 vaccine by subcutaneous and intracaudal routes. The two vaccinated groups together with an untreated control group were exposed to a single ineffective dose of virulent *Brucella abortus* organisms through conjunctiva during their first pregnancy.

The intracaudal route had been shown, previously, to be more efficient for agglutinin production, than either the subcutaneous or intradermal routes, using a lesser dose. The other advantages claimed are that the inoculation into the tip of the tail presents little technical difficulties, and there is freedom from severe and persistent local reactions. Also there is economy in the use of the vaccine.

The present studies in this paper were undertaken—(1) to obtain further evidence on the claim that the intracaudal route is superior to subcutaneous route in inducing serological responses; (2) to determine if some correlation could be established between serological response after vaccination and immunity to infection and abortion; and (3) to determine if the intracaudal vaccination of heifer calves, during calfhood with 1 c.c. of strain 19 vaccine did induce a higher degree of resistance to infection as compared with the subcutaneous method of vaccination.

A preliminary experiment, in this paper, demonstrates a highly significant superiority of the intracaudal route over the subcutaneous route, in inducing serological responses of unmated yearling heifers.

No correlation could be established between the serum agglutinin titres of the vaccinated animals, two weeks after inoculation, with the length of the gestation period, thus suggesting that agglutinin response cannot always be accepted as a measure of immunity.

Also, no significant difference could be demonstrated in the infection or abortion rates, percentages of live calves or mean lengths of gestation periods. This might suggest that the intracaudal method of vaccination of heifer calves is as efficient as subcutaneous method.

The author concludes by saying that under conditions of this experiment—Intracaudal calfhood vaccination of heifer with cotton strain 19 failed to demonstrate a higher degree of resistance to infection or abortion than that shown by a similar group of animals immunized by the subcutaneous method, when exposed to infection during their first pregnancy. [M. S. J.]

The feeding of gammexane and D. D. T. to bovines.
WILLIAM, S. G. (1938). *Bull. Entomol. Res. 39*, 423-434

A SERIES of experiments with oral feeding of Gammexane D. 929 showed that dose of 0·5 gm./kgm. on two successive days proved rapidly toxic to bovines. The main toxic symptom exhibited by the animal were, hypersensitivity of the skin muscular tremors, general paralysis followed by death. A dose of 0·25 gm./kgm. on two successive days followed by 0·125 gm./kgm. on 3rd and 4th days was well tolerated and killed feeding *Glossina palpalis* and ticks. This dosage protected the calves against re-infestation of ticks for 41 days.

Two calves given four weekly doses of 0·25 gm./kgm. and 0·125 gm./kgm. were protected against re-infestation of ticks for a period of 20 days and 12 days respectively where as weekly doses of 0·1 gm./kgm. have failed to produce any ticki-cidal effect.

A D D T powder (containing 83 per cent of the para-para Isomer), when fed to calves in doses of 0·5 gm./kgm. and 0·25 gm./kgm. respectively failed to kill feeding *Glossina palpalis*. [D. N.]

Beneficial effect and economic importance of using all colostrum produced in calf raising. KAESER, H. E. and SUTTON, T. S. (1948). *J. Dairy Sci.* 31, 523-532

THE authors' investigations were to determine the effects of intermittent colostrum feeding for the duration of milk feeding period of six months. The term Colostrum is used for the first three days secretion immediately following parturition. The high nutritive value of colostrum for the new-born calf is well known. The vitamin A content of colostrum is many times that of normal milk. It is also high in riboflavin which is also essential for the calf. Yet with all these qualities and knowledge the surplus colostrum is not used for calf raising in many dairies.

Seventy-six calves born in the Ohio State University dairy herd during one year period from five different major breeds were used for observations. The calves of either sex were divided into two comparable groups—36 in group I and 40 in group II. 22 of the 36 in group I remained for the full term of 24 weeks and 20 out of the 40 in group II. Calves were weighed at birth, 3rd day and later weekly during the experimental period. They were bled for Vitamin A and carotene analysis at the time of weighing during the first four weeks and once a month later.

All the calves received colostrum for the first 3 days from their dams by nipple-pail feeding. Later the controls (group II) was fed whole Holstein milk at 10 per cent of their body weight for the first 3 months, 8 per cent during the fourth month, 6 per cent during the fifth month and 4 per cent during the sixth month. The experimental calves (group I) received the same quantity but the Holstein milk was replaced part or all when colostrum was available. All calves received a concentrate mixture, mixed hay of average quality and water *ad libitum* up to 3 months of age and later the concentrate was increased and fed twice daily and at 6 months they were getting 4 lb. daily.

Calves in group I *i.e.* colostrum fed maintained high blood plasma levels for vitamin A and carotene, increased in weight more rapidly and had a better healthy appearance especially during the first two months. No digestive disturbances were

noticed directly due to extra colostrum feeding. The amount of colostrum received by each calf, the blood plasma vitamin A and carotene level and the gain in body weight are all tabulated. It was found that calves receiving more than 200 lb. of colostrum maintained higher levels of vitamin A in the blood plasma and made greater cumulative weight gains than those received less than 200 lb. During this experiment over 4000 lb. of surplus colostrum was used in feeding the calves in group I and thus same quantity of marketable milk was saved. It is estimated that even if half the surplus colostrum from the 26 million dairy cows in the U.S.A. is utilized in calf raising it will save 650 million lb. of marketable milk. The surplus colostrum can also be frozen and stored wherever it is possible and used when required. Thus the economic importance of using all colostrum in raising calves has been fully demonstrated by these experiments. [M.K.S.]

Breeding chickens for resistance to caecal coccidiosis. Palafox, A. L., Alicata, J. E. and Kaxtman, L. (1949). *World's Poult. Sci. J.* 5, 2, 84-87

THE authors started breeding work with the foundation stocks comprising White Leghorn hens having the highest rate of survival in progeny against caecal Coccidiosis and a susceptible line of the same breed having the lowest rate of survival of progeny. In breeding of these two lines revealed that the dams selected for high resistance to Caecal Coccidiosis transmitted high resistance to their F_1 progeny. Similarly dams selected for low resistance transmitted only low resistance to their progeny. Resistance or susceptibility in the F_1 progeny was tested experimentally at four weeks of age by inoculating with approximately 120,000 sporulated oocysts of *Eimerla tenella*. However, in the resistant line the individual variation of the degree of resistance in the progeny of resistant dams mated to the same sire was considerable indeed.

In another attempt reciprocal matings between the resistant and susceptible lines showed that neither line was homozygous for the character. Further selection amongst resitant birds was therefore indicated to obtain homozygosity.

In the F_2 progeny of the resistant line increased resistance was demonstrated whereas in the unselected control lot it was intermediate between the resistant and susceptible lines. It is suggested, therefore, that resitance to Caecal Coccidiosis is dependent, at least in part, on inherited multiple factors.

Coccidiosis amongst rearing stock in specialised farms in India is all too common. From the foregoing results it seems breeding chickens for natural resistance to Coccidiosis is a profitable line of approach to the problem of solving poultry mortality. [S.G.I.]

The Galactopoietic effect of Iodinated Casein : Dose Response Relationships during Prolonged Treatment. Leech, F. B. (1950). *J. Endocrinology*, 7, 42-53

FIFTYNINE cows were fed daily with iodinated casein in different dosage levels of 15, 20, 25 g. for 28 weeks from 3 months after calving except in cows which become dry before time. The increase in milk yield lasted for a period of 8 weeks after which the response to the two lower doses quickly disappeared. The response of the group receiving 25 g. of iodinated casein was maintained at a

reduced level throughout. The treatment did not effect the proportions of butter fat and non-fatty solids in the milk. There was some effect on the heart rate but in general the cows showed no other symptoms indicative of *Hyper thyroidism.* Loss in body weight was directly proportional to the dose administered. This loss in weight gradually disappeared. There was no significant effect on the health of the animals or on their reproductive activity. Long continued use of thyroactive materials is not recommended. The probable role of thyroxine in augmenting the milk yield has been discussed. [S. K. De.]

Comparative Nutrition of Farm Animals. H. R. Guilbert and J. K. Loosli (1951) *J. Anim. Sci.* **10**, 22-41

THE present report on the comparative nutrition of farm animals was undertaken by the authors in view of the discrepancies, pointed out by Maynard in the feeding standards for livestock recommended by the Committee on the Animal Nutrition of the National Research Council, in 1942.

The relevant literature, and the concepts contributing to the procedure adopted by the authors, have been reviewed. The concept of physiologically equivalent age has been used to compare the recommended nutritional requirements of different species of animals that fit with the physiologically equivalent age curve.

The total feed capacities of the different species are in general, *proportional* to the fractional power of bodyweight as the maintenance requirement and the basal heat production. There is no fundamental difference in the efficiency of feed utilization for growth, that depends on body size as such. There are no differences in the recommended dietary allowances for protein, calcium, phosphorus for different species but the ratio of these nutrients to energy intake changes with advancing age. The recommended requirements for thiamine, riboflavin, niacin, and pantothenic acid, indicated a constant direct relation with intake of energy irrespective of the different stages of development, and is consistent with their function in enzyme systems associated directly or indirectly with the metabolic rate. It is also pointed out that the vitamin requirements of vitamin A and carotene are directly proportional to the bodyweight in mammals. This applies to poultry, as well, but the requirement is somewhat higher than mammals. The existing knowledge about the nutritional requirements of vitamin D, would point out to a lack of any relation either with the body weight or feed consumption.

The generalized recommended nutritional requirements for farm animals, as a per cent of the total digestible nutrients in relation to per cent mature weight, is presented, which with the existing knowledge or the results of future research is capable of being translated into suitable feeding standards for practical use.

The authors suggested an investigation into the feasibility of expressing the relationship of the various nutrients with the metabolizable energy rather than with the total digestible nutrients in order to bring the data for ruminants and non-ruminants into closer accord. The field of comparative nutrition is fertile and there is ample scope for studying the similarities, which are more likely to be more pronounced, than differences, that exist among the different species of farm animals. [V. N. M.]

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ORIGINAL ARTICLES

SEASONAL VARIATIONS IN SEMEN QUALITY, AND HAEMOGLOBIN AND CELL VOLUME CONTENTS OF THE BLOOD IN BULLS

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(With Plates IV—V)

IN farm animals spermatogenetic activity goes on continuously in the male after the attainment of puberty and they are capable of mating throughout the year. In recent years, however, many investigators have shown that the quality and quantity of semen in certain species of farm animals vary during different seasons of the year. Most of these studies have been carried out in Western Countries. In India, Shukla and Bhatacharya [1947] have shown seasonal variations in the semen quality of indigenous sheep and goats.

In bulls Weatherby *et al.* [1940] found slight monthly variations in volume, sperm concentration and longevity of spermatozoa in bull semen. Anderson [1941] reported seasonal variation in volume and motility of spermatozoa only. Erb *et al.* [1942] reported highly significant difference between seasons in all the factors of bull semen studied, except *p*H. Phillips and his co-workers [1943,*a*] observed significant or highly significant variations in all the characteristics of semen except in volume and motility of spermatozoa. Lasley and Bogart [1943] reported that beef type bulls produce semen of high quality between May to September. Swanson and Herman [1944] found seasonal variation in *p*H, initial motility, viability and abnormality of spermatozoa. Salisbury [1944] observed seasonal trend in motility and sperm concentration. Anderson [1945,*b*] and Mercier and Salisbury [1946] carried out systematic studies on the effect of season on semen attributes Anderson observed highly significant monthly differences in sperm concentration, motility and *p*H of semen. The latter authors obtained highly significant month to month variations in sperm motility, concentration abnormal sperms, methylene blue reduction time and fertilising capacity of semen and significant variation in volume but not in total number of spermatoza and motility.

A fact which clearly emerges out of this review is the lack of uniformity in the results obtained by the various authors. This may be due particularly to the different climatic conditions under which the experiments were carried out. Difference in breeds, age, management and nutrition might also be contributory factors. The quality and quantity of semen are dependent on all the factors enumerated above. It is, therefore, important that in a study relating to seasonal effect on semen, the various factors which may have any influence on the

characteristics of semen should be uniform and controlled. Unless sexually mature animals of the same age and breed are taken for experimentation and are kept under uniform managerial and dietary regime during the entire experimental period, seasonal influence, if any, on the various characteristics of semen may be masked, minimised or exaggerated.

Indian bulls (*Bos indicus*) belong entirely to a different species from the experimental animals of the previous workers. Climatic conditions in this country also are greatly different from those in places where the experiments were conducted. For these reasons and in view of the contradictory results obtained by the various workers it was considered worth while to study the trend of seasonal influence on semen characteristics of Indian bulls.

Seasonal variations in the composition of blood of Indian dairy cattle have been observed by Pal *et al.* [1945]. Attempts have, therefore, been made in this study to determine the relationship, if any, of the seasonal changes in the blood with the variations in the semen characteristics.

Material and methods

The experimental animals comprised of six Kumauni hill bulls. All the bulls were of four years of age excepting one (No. 187) which was seven years old at the commencement of the experiment. During the entire experimental period the bulls were kept under uniform managerial and dietary regime. The ration supplied to each bull consisted of a concentrate mixture containing crushed gram 30 parts, crushed barley 50 parts, wheat bran 20 parts and common salt 1 oz. Concentrate mixture was fed according to body-weight. The quantity given per day to the bulls was as follows :

Bull number	Average body weight	Concentrate given
200	500 lb.	4 lb.
187 and 240	450 lb.	3 lb.
194,195 and 248	400 lb.	2 lb.

In addition to the above concentrate each animal received 10-lb. of roughage and was allowed free grazing for six hours a day.

The bulls selected were in good health having good sex desire and sex ability. During the pre-experimental period they were trained to mount an anoestrous hill cow confined in a service crate and to ejaculate semen in the artificial vagina.

In order to estimate the mo[illegible] [illegible]ariations in semen quality and quantity of individual bulls, fortnightly co[illegible] of two successive ejaculates at an interval

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In order to estimate the monthly variations in semen quality and quantity of individual bulls, fortnightly collections of two successive ejaculates at an interval

of 10 to 15 minutes were made from each bull. Each sample of semen was examined for the following characteristics :—

(1) Colour and consistency.

(2) Volume.

(3) Initial motility of spermatozoa.

(4) Initial pH of semen.

(5) Sperm concentration per c.c. of semen.

(6) Total number of spermatozoa per ejaculate.

(7) Percentage of abnormal spermatozoa.

Observations of each sample for its colour and consistency, volume, pH and initial motility were made immediately after collection. Colour and consistency were determined by the visual appearance of the semen ; volume was measured to the nearest 1/100 of c.c. ; the degree of motility was scored in terms of criteria as recommended by Erb *et al.* [1948] ; pH was determined by means of B. D. H. capi lator and sperm concentration was determined by the usual haemacytometor method in terms of millions per c.c.

To determine the percentage of abnormal spermatozoa the following method of fixation and staining was adopted. A thin film of diluted semen in normal saline was made on a slide. Spermatozoa in the film were then fixed in the vapour of two per cent. osmic acid solution for about 15 minutes. Care was taken to expose spermatozoa in the film to osmic vapour within 5 to 10 minutes after collection of the semen sample. The film was then allowed to dry in the room temperature and was later stained with 0·2 per cent. aqua solution of methyl violet for 20 to 25 minutes, washed in tap water and dried. One hundred spermatozoa were examined at random under oil immersion lens and the number of various types of abnormal spermatozoa were noted.

Besides the above mentioned semen characteristics, the 'reaction-time' of the animals at the time of collection of semen was also noted. The 'reaction-time' which expresses the degree of sex vigour was determined by the time interval between the release of the bull near the cow and the actual moment of ejaculation.

A blood sample was also drawn once every fortnight from the jugular vein of each bull for determining the haemoglobin and cell volume contents. The sample of blood was drawn on the day following the collection of semen. Haemoglobin percentage was determined by New Comer's method [1923] and cell volume by the method of Napier and Das Gupta [1941].

TABLE I

Monthly averages of 'reaction-time', semen and blood characteristics of the bulls and the meteorological data

	1945-46													
	J	F	M	A	M	J	J	A	S	O	N	D	J	F
Reaction-time (in seconds)	91·46	40·63	65·63	69·12	69·73	117·50	48·58	81·71	97·0	52·29	141·75	95·83	62·96	82·13
Appearance of semen	M	M	M	M	Th. M	Th. M	Th. M	Th. M	Th. M	M	M	M	M	M
Initial motility of spermatozoa	4·19	3·85	3·63	3·65	3·54	3·08	3·48	3·21	3·75	3·69	3·75	3·92	3·23	3·38
Volume of semen (in c.c.)	2·05	2·52	2·22	2·03	2·17	2·40	2·44	2·33	2·00	1·93	1·91	1·87	2·27	2·03
pH of semen	6·18	6·15	6·32	6·3	6·21	6·24	6·32	6·11	6·12	6·19	6·24	6·23	6·51	6·52
Sperm concentration (in millions per c.c. of semen)	698·54	625·0	788·33	658·33	591·66	532·29	564·79	511·70	561·87	609·08	680·88	676·88	720·0	698·13
Total number of spermatozoa (in millions)	1,318·67	1,549·51	1,772·95	1,417·39	1,338·65	1,467·31	1,492·77	1,275·68	1,178·99	1,165·85	1,296·33	1,309·17	1,713·84	1,482·89
Percentage of abnormal spermatozoa	4·63	5·88	5·71	5·13	2·63	3·71	2·58	4·38	4·79	8·63	5·33	5·63	9·25	7·00
Haemoglobin (gm. per 100 c.c. of blood)	8·79	8·91	8·82	8·89	8·77	8·29	7·89	7·82	7·37	7·63	7·88	8·12	8·90	8·85
Percentage of cell volume	45·65	45·48	43·49	42·92	43·27	40·04	38·14	32·99	35·52	36·96	37·56	42·01	45·33	43·99
Air temperature (°F.)	60·9	67·1	73·3	84·8	88·5	89·5	83·7	84·9	82·7	77·1	68·6	59·1	58·9	64·2
Percentage of humidity	59	61	34	42	47	61	85	79	83	68	66	67	71	63
Rainfall (in inches)	0	0	0·01	0·68	1·72	4·59	22·21	8·59	13·94	5·03	0	0	1·32	0·75

M=Milky Th. M=Thin milky

during the entire experimental period

1946-47									
M	A	M	J	J	A	S	O	N	D
33·46	112·33	36·17	30·0	76·46	103·28	54·67	51·53	78·21	91·88
M	M	M	M	M	Th. M	Th. M	Th. M	M	M
3·38	3·38	3·42	3·13	3·10	3·56	3·29	3·40	3·54	3·27
2·24	2·20	2·00	2·02	2·31	2·82	2·33	2·30	2·49	2·14
6·29	6·20	6·26	6·33	6·37	6·33	6·34	6·34	6·33	6·48
704·79	720·42	665·63	670·83	636·00	569·58	500·42	456·46	711·04	676·42
1,422·13	1,450·25	1,272·13	1,195·84	1,428·36	1,834·62	1,180·97	1,069·31	1,721·14	1,386·99
3·96	5·79	6·33	5·04	3·25	5·63	3·96	5·75	5·75	6·33
8·76	9·34	9·59	8·70	8·25	7·90	7·42	8·18	8·24	8·07
41·76	44·95	45·95	42·67	40·13	38·14	38·99	40·73	41·55	41·03
75·3	86·7	92·7	93·5	85·6	85·9	85·4	79·0	68·9	61·7
45	22	31	50	81	80	65	72	64	62
0·96	0·00	1·01	5·25	16·89	6·11	4·01	5·17	0·04	0·21

RESULTS

Data regarding the average 'reaction-time', semen and blood attributes of the bulls together with the meteorological data of each month are presented in Table I. Examination of the data reveals that there did not exist any relationship between the 'reaction-time' and the quality of semen produced by the bulls in each month. Average 'reaction-time' in 1945-46 was the shortest in February and longest in November. There was, however, no appreciable difference between these months in majority of the semen characteristics. In 1946-47 the 'reaction-time' was the lowest in June and highest in April. The over-all quality of semen during these two months of that year were diametrically opposite; semen samples of April were decidedly superior to June samples.

Colour of the semen samples produced by the bulls during the experimental period were either yellow or milky white. Yellow samples were the characteristic feature of the bull 187. They were thin in consistency and were of low sperm concentration. The semen samples produced by the remaining five bulls were generally milky white in colour. The consistency was either very thin giving a cloudy appearance or like that of milk, or thick giving a milky creamy appearance. Creamy samples, of which there were a few, contained very high concentration of spermatozoa. Milky samples were of higher sperm concentration than cloudy or yellow samples.

In working out the average colour and consistency for the month as presented in the table, the characteristics of the majority of the samples for the month were only considered. From the table it is apparent that sperm concentration of thin milky samples ranged from 456·5 to 591·7 millions per c.c. and that of milky samples from 609·1 to 788·3. There was no marked monthly variation in the initial motility of spermatozoa and the volume of semen. The latter, however, showed some decrease from October to December during the first year of the experiment.

No marked monthly variation was noticed in *p*H. The average *p*H value of semen samples during the first year was lower than that of the second year.

In 1945-46, sperm concentration and haematological constituents decreased from May to September and in 1946-47 from July to October. Meteorological data of these periods show high air temperature associated with high relative humidity and rainfall.

Total number of spermatozoa was calculated by multiplying the total volume with the sperm concentration. Inspection of the data presented in the table shows that in this characteristic there existed variations from month to month. These variations were statistically analysed. The implication of the analysis has been discussed later on. In the first year, percentage of abnormal spermatozoa was minimum during May to July and maximum during October. In the second year it dropped down to minimum in March, July and September and maximum was reached during January.

Average monthly variations in 'reaction-time', in semen characteristics and in blood constituents are presented in Plate IV, and that of air temperature, relative

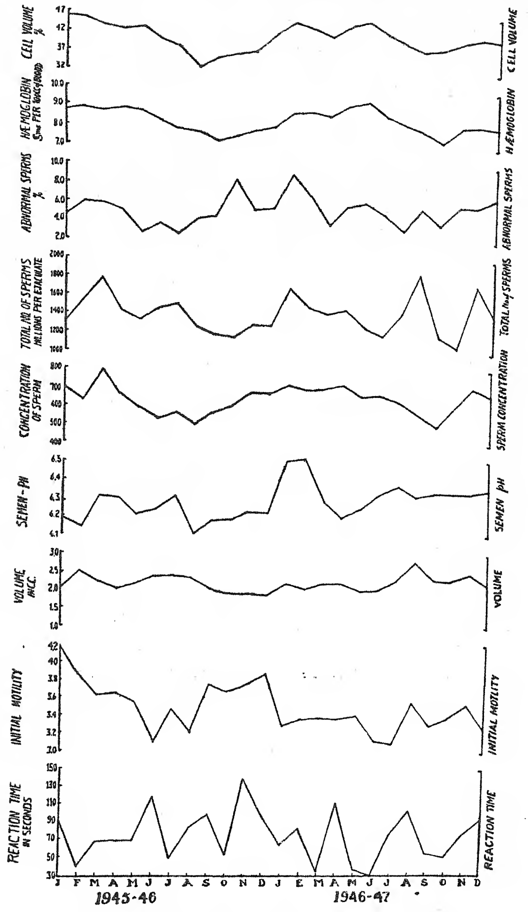
humidity and rainfall in Plate V. From Plate IV it is apparent that the curve for 'reaction-time' is not uniform and does not show any definite seasonal trend. Initial motility curve shows a tendency to follow the curve for sperm concentration. The curve for volume reached its peak in August 1946 and minimum in December 1945-46. The curve for pH shows an opposite trend to that of sperm concentration. The curves for sperm concentration, haemoglobin and cell volume run parallel to one another and show definite seasonal trends. From Plate V it is evident that with the rise of air temperature, relative humidity and rainfall, there was decrease in the sperm concentration and in the two blood constituents studied. Low air temperature associated with high humidity as found in the month of December of both the years had depressing effect on sperm concentration and blood constituents. The curve for total number of spermatozoa does not uniformly resemble the curve representing sperm concentration. The curve for abnormal spermatozoa has two peaks, one in October 1946 and the other in January 1947.

TABLE II

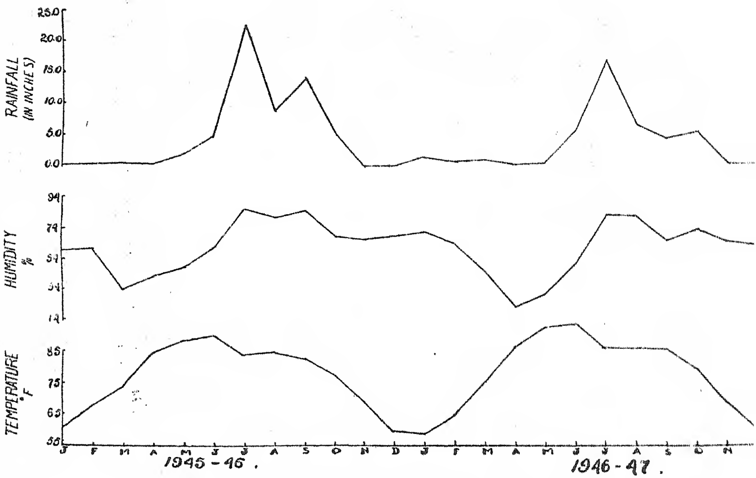
Average 'reaction-time', semen and blood characteristics of the bulls and the meteorological conditions in different seasons

	Autumn (August to October)	Winter (November to January)	Spring (February to April)	Summer (May to July)
Reaction time (in seconds)	73·42	93·63	67·23	63·07
Colour and consistency of semen	Th. M.	M.	M.	M.
Volume of semen (in c.c.)	2.28	2·12	2·21	2·22
Initial motility of spermatozoa	3·48	3·60	3·53	3·29
pH of semen	6·25	6·32	6·29	6·28
Sperm-concentration (in millions per c.c.)	534·85	693·96	699·17	610·20
Total number of spermatozoa (in millions)	1,284·24	1,457·86	1,515·85	1,365·85
Percentage of abnormal spermatozoa	5·52	6·15	5·58	3·92
Haemoglobin contents in blood (grams per 100 c.c. of blood)	7·72	8·33	8·93	8·58
Percentage cell volume	37·22	42·19	43·76	41·70
Percentage of humidity	74·5	64·83	44·5	59·16
Rainfall (in inches)	7·14	0·26	0·40	8·45
Air temperature (0°F.)	82·95	62·95	75·23	88·91

Th. M.=Thin milky
M.=Milky



Monthly variations in 'reaction-time', semen and blood characteristics.



Monthly variations, in average air temperature, humidity and rainfall.

Table II shows variations in average 'reaction-time', semen and blood characteristics of the bulls and the meteorological conditions during the four seasons. The figures given in the table are the averages for two consecutive experimental years. Perusal of the table reveals that there did not exist any correlation between the 'reaction-time' and the quality of semen in different seasons. 'Reaction-time' was longest in winter and shortest in summer. Very little difference in volumes and pH of semen existed during the seasons. Sperm concentration, total number of spermatozoa, haemoglobin and cell volume contents of blood were lowest in autumn and highest in spring. Initial motility of spermatozoa was lowest in summer and highest in winter. Percentage of abnormal spermatozoa was conspicuously low in summer. Relatively, little difference was observed among the remaining three seasons. In winter and spring when the semen was rich in sperm concentration, the samples had a milky appearance. In summer and autumn the ejaculates contained lesser number of spermatozoa and their appearance varied from milky to thin milky. Relative humidity and rainfall were highest in autumn and lowest in spring. Average air temperature in autumn was almost as high as in summer. Air temperature in spring was moderate.

Statistical analysis of the results

The data summarised in Table I and II, and the average quality of semen produced by the individual bulls during the entire experimental period have been subjected to analysis of variance to determine whether or not the variations as found in semen qualities, blood constituents and meteorological data were real or due to chance. The summary of the results of the statistical analysis has been presented in Table III.

TABLE III

Summary of the analysis of variance of semen and blood characteristics of the bulls and meteorological data

Factors for analysis	Source of variation				
	Between bulls	Between years	Between seasons	Interaction between seasons and years	Between months within seasons
Reaction-lime	**	०	०	०	०
Volume of semen	**	०	०	०	०
Initial motility of spermatozoa	**	**	०	०	०
pH of semen	**	**	०	०	**
Sperm-concentration	**	०	**	०	०
Total number of spermatozoa	**	०	०	०	०

TABLE III—*contd.*

Factors for analysis	Source of variation				
	Between Bulls	Between years	Between seasons	Interaction between seasons and years	Between months within seasons
Percentage of abnormal spermatozoa	°	°	**	°	°
Percentage of haemoglobin	**	**	**	°	**
Percentage of cell volume	**	**	**	*	**
Average humidity	..	°	*	°	°
Average rainfall	..	°	*	°	°
Average temperature	..	°	**	°	°

**Significant at one per cent level.

*Significant at five per cent level.

°Non-significant.

The results show that in all the characteristics of semen and blood, excepting in the percentage of abnormal spermatozoa there was highly significant variation among bulls. In other words, the variations observed were so great that differences due to chance were less than one per cent. Between the two experimental years of 1945-46 and 1946-47, the semen characteristics observed, excepting the initial motility of spermatozoa and *p*H of semen, were due to chance variations. Haemoglobin as well as cell-volume contents of blood were highly variable between years. Among seasons, variability in initial motility was just significant, whereas the variations in sperm concentration, percentage of abnormal spermatozoa, haemoglobin and cell-volume were highly significant. Cell-volume varied due to interaction of seasons and years. Among months within seasons, highly significant variations were found in *p*H of semen and blood characteristics.

Analysis of variance of the meteorological data was carried out between years among seasons and interaction between seasons and years. It will be seen from the table that air temperature variation among seasons was highly significant. There was, however, significant variation in relative humidity and rainfall.

To test the differences between significant and highly significant factors as presented in Table III, critical difference (C.D.) of the variable factors at 5 per cent level of probability was worked out and the results are presented in Tables IV to VI. In each of the tables average figure under the same bar did not vary at 5 per cent level. Table IV shows C. D. of variable factors among bulls.

TABLE IV

Critical difference of five per cent level of variable factors between bulls

	Value of C.D.	Averages of bulls arranged in ascending order					
Reaction-time (in seconds)	36·81	240 (42·17)	194 (52·64)	248 (57·67)	187 (83·23)	195 (100·43)	200 (109·94)
Volume of semen (in c.c.)	0·34	248 (1·56)	195 (2·07)	194 (2·10)	200 (2·11)	240 (2·43)	187 (3·00)
Initial motility	0·27	187 (2·55)	248 (3·04)	194 (3·73)	200 (3·75)	240 (3·86)	195 (4·02)
pH of semen	0·08	195 (6·21)	240 A(6·22)	200 (6·24)	187 (6·33)	194 B(6·34)	248 (6·39)
Sperm concentration (in millions per c.c.)	114·92	187 (308·95)	248 (410·20)	200 (628·44)	240 (757·76)	195 (806·05)	194 (895·83)
Total number of spermatozoa per ejaculate (in millions)	318·74	248 (690·44)	187 (1,041·35)	200 (1,351·35)	194 (1,724·33)	195 (1,745·32)	240 (1,882·65)
Percentage of haemoglobin (in gm. per 100 c.c. of blood)	0·24	187 (8·04)	200 (8·06)	248 (8·12)	195 (8·34)	240 (8·70)	194 (9·10)
Percentage of cell volume	1·49	187 (39·12)	248 (40·25)	200 (40·94)	195 (41·10)	240 (42·90)	194 (43·90)

TABLE V

Critical difference at 5 per cent level of variable factors between years

	Value of C.D.	Averages of years arranged in ascending order	
Initial motility of spermatozoa	0·12	1946-47 (3·33)	1945-46 (3·64)
*p*H of semen	0·04	1945-46 (6·22)	1946-47 (6·36)
Percentage of haemoglobin	0·14	1945-46 (8·27)	1946-47 (8·52)
Percentage of cell volume	0·87	1945-46 (40·37)	1946-47 (42·10)

TABLE VI

Critical difference at 5 per cent level of variable factors between seasons

	Value of C.D.	Averages of seasons arranged in ascending order			
Initial motility	0·22	Summer (3·29)	Autumn (3·48)	Spring (3·54)	Winter (3·66)
Sperm concentration per c.c. of semen.	93·84	Autumn (534·85)	Summer (610·20)	Winter (693·96)	Spring (699·17)
Percentage of abnormal spermatozoa.	1·24	Summer (3·92)	Autumn (5·52)	Spring (5·58)	Winter (6·15)
Percentage of haemoglobin in blood.	0·20	Autumn (7·72)	Winter (8·33)	Summer (8·58)	Spring (8·93)
Percentage of cell volume	1·21	Autumn (37·22)	Summer (41·70)	Winter (42·19)	Spring (43·76)
Percentage of humidity	18·66	Spring (44·50)	Summer (59·16)	Winter (64·83)	Autumn (74·50)
Average rainfall	6·40	Winter (0·26)	Spring (0·40)	Autumn (7·10)	Summer (8·45)
Average air temperature	7·86	Winter (62·95)	Spring (75·23)	Autumn (82·50)	Summer 88·91)

It is apparent from the table that the 'reaction-time' of bulls did not show any relationship with the quality of semen produced by them. The quality of semen of bulls under the same bar, which did not show significant variation in their average 'reaction-time' varied considerably. For instance, the semen quality (as judged by initial motility, sperm concentration and total number of spermatozoa) of bull 248, whose 'reaction-time' did not vary significantly from bulls 240 and 194, was significantly inferior to that of those two bulls. Bulls 195 and 200 did not vary in their 'reaction-time' but there was a significant variation in all their semen attributes except *p*H, motility and volume of semen; bull 200 was inferior to bull 195. Similarly, the semen of bull 187 which did not significantly vary from bull 194 as regards 'reaction-time', was inferior to this bull. It seems, therefore, that in bulls the 'reaction-time' which is an indication of sex-vigour is independent of sperm production and the quality of semen.

Regarding volume of semen, bull 187 gave the largest quantity and bull 248 the smallest. Bull 195 was inferior to bull 240. The rest of the bulls did not vary among themselves. Although the volume of semen from bull 187 was much higher than from the rest, the initial motility and sperm concentration in this bull were the lowest. It seems that the amount of the accessory glands secretions in this bull was much more than in others, presumably it was due to this particular animal being three years older than the rest.

Initial motility of bull 248 was significantly higher than bull 187 but lower than the remaining bulls. Bull 194 was inferior to bull 195. The variation among bulls 194, 200 and 240 was not significant.

As regards *p*H of semen there were two distinct groups. Group A included bulls 195, 240 and 200 and Group B included 187, 194 and 248. The semen quality (as judged by sperm concentration, initial motility and total number of spermatozoa) of all the animals in Group A was decidedly superior to that of bulls 187 and 248 of Group B. The reason why the semen of bull 194 showed higher *p*H inspite of higher concentration and total number of spermatozoa may be found in the fact that during December 1946 to April 1947 the animal suffered from inflammation of the seminal vesicles. Due to this inflammation the semen samples during this period were found to be very alkaline and contained leucocytes; furthermore the initial motility of spermatozoa was also low during this period. It appears, therefore, that in animals showing the normal range of *p*H of semen, those with comparatively higher *p*H value are poorer in sperm concentration, total number of spermatozoa and initial motility.

Sperm concentration in bull 200 was significantly higher than in bulls 187 and 248. Bull 240 was inferior to bull 194. It will be seen that there was a direct relationship between the sperm concentration and initial motility of spermatozoa. Sperm concentration and initial motility were observed to be lower in bull 187. Bull 200 which was decidedly better than bulls 187 and 248 for sperm concentration gave samples with higher initial motility. Between bulls 240 and 195 there was neither significant variation in sperm concentration nor in initial motility.

As has been stated before the total number of spermatozoa was calculated by multiplying the density of the ejaculate by its volume. It was expected, therefore, that in bulls, showing significant differences in volume but not in sperm concentration the total number of spermatozoa with larger volume of ejaculate would be higher. This was found to be true in bulls 248 and 187. Sperm-concentration of these two bulls did not vary. However, as bull 187 was much superior to bull 248 in volume, the total number of spermatozoa was significantly greater than that of 248 which had the lowest semen volume. There was no significant variation in the total number of spermatozoa between bulls 187 and 200. This was perhaps due to the fact that the volume of semen of the latter was significantly less than that of the former although its sperm concentration was significantly higher. Bulls 194 and 195 did not vary in this characteristic as there was neither significant variation between their semen volumes nor between their sperm concentrations. Between bulls 195 and 240 there was not significant variation in sperm concentration. Although there was a significant variation in their volume of semen, the variation was not so great as between bulls 187 and 248. It was prehaps due to this reason that no significant variation in the total number of spermatozoa between bulls 195 and 240 was observed.

Regarding the blood constituents it will be seen from the table that there was a very close relationship between cell volume and haemoglobin contents. Bulls having higher concentration of haemoglobin also had higher cell volume in blood. Judged by these two characteristics, the quality of blood of bull 187 was poorest. Furthermore, the semen quality of this bull as regards initial motility and sperm concentration was very poor and the pH of semen was high. Bull 248 was significantly inferior to bulls 240 and 194 for blood as well as for semen attributes. Bull 194 which showed highest concentration of haemoglobin and cell volume in blood also showed highest sperm concentration in semen. Its total number of spermatozoa did not vary significantly from that of bull 240 which showed the highest number of spermatozoa.

From the records of bulls 187, 194 and 248 it appears that there was a direct correlation between semen and blood quality of bulls. Had it been really so, bulls 200 and 248 which did not vary significantly in their blood constitutions should not have varied in their semen quality as well. But bull 248 was the poorest of all for volume and total number of spermatozoa. The pH value of semen was also high and there was not significant variation in sperm concentration between 248 and 187 which had the lowest sperm concentration. Similarly, bull 195 did not vary significantly in blood picture from bull 248 but its semen quality was decidedly superior to bull 248.

Table V shows C. D. at 5 per cent level of variable factors between years. From the table it will be seen that during the first year of the experiment the initial motility of spermatozoa was significantly higher than in the second year, whereas, the pH value in the second year was more than in the first year. Blood samples of the animals during 1946-47 were richer in haemoglobin and cell volume than in 1945-46. It is apparent, therefore, that although the general conditions of the

animals improved during the second year as shown by higher haemoglobin and cell volume contents of blood, their semen quality deteriorated as indicated by higher *p*H value of semen and lower initial motility of spermatozoa. No satisfactory explanation for this deterioration in semen quality seems possible without detailed studies of the various contributory factors for semen production.

Critical difference of the variable factors between seasons is given in Table VI. From the table it is apparent that there was no significant variability in the initial motility and sperm concentration between summer and autumn. For initial motility summer and for sperm concentration autumn was the worst season. For haemoglobin and cell volume in blood, autumn was decidedly inferior to the rest of the seasons. Haemoglobin varied from season to season but no significant variation in cell volume was found between winter and summer. For the two blood constituents and for sperm concentration, autumn was the worst and spring the best season. Autumn differed significantly from spring in relative humidity and rainfall; these in the former season being 30·0 per cent and 6·74 inches respectively more than in the latter. There was, however, no significant variation in the average air temperature between the two seasons. It appears, therefore, that sperm concentration in semen as well as the two blood constituents improved considerably when the environmental temperature was moderate and humidity low.

TABLE VII

Percentage distribution of abnormal spermatozoa in different seasons

Seasons	Abnormalities of head	Abnormalities of middle piece		Abnormalities of tail			
		Beaded middle piece	Other abnormalities	Tailless	Bent tail	Coiled tail	Other abnormalities
Autumn	2·66	2·7	0·95	12·65	9·33	2·54	2·46
Winter	2·83	2·04	0·25	8·33	15·75	3·46	2·91
Spring	2·12	2·33	0·25	8·37	12·33	3·16	4·66
Summer	2·66	2·25	0·45	11·08	3·91	1·83	1·79

As regards abnormal spermatozoa, summer differed significantly from the rest of the seasons. In summer percentage of abnormal spermatozoa was low. Percentage of abnormal spermatozoa during the different seasons of the year is given in Table VII. The most common types of abnormalities have been tabulated under different heads and those which were not common have been put under one group 'other abnormalities'. It will be seen from the table that in winter and spring 'bent tail' spermatozoa increased considerably, whereas autumn and summer favoured

the formation of tailless ones. Percentage of 'coiled tail' spermatozoa was higher in winter and spring and lower in autumn and summer. The rest of the abnormalities did not show any marked variation from season to season. The fall in the percentage of abnormal spermatozoa in summer might be due to the fact that during this season the 'bent tail' sperms were minimum although the tailless ones were as high as in autumn.

Consideration of critical difference of pH and blood constituents between months has not been attempted as the samples obtained from each bull in a month were too small to yield any valid conclusion.

Discussion

As has been stated above, statistical analysis of the data on 'reaction-time' revealed that it was not influenced by season. Salisbury [1944] also did not find any significant monthly variation in bulls with regard to this character.

'Reaction-time' is an indication of sex desire or *libido* and sex vigour. Sex desire as judged by 'reaction-time' varied considerably in bulls, but it did not give any indication as regards their sperm production. This observation is in agreement with those reported by McKenzie and Berliner [1937] in rams, and Anderson [1939] in bulls.

Various workers have shown that sex desire in males is controlled by male hormone (testosterone). Injection of testosterone to immature, castrated or hypophysectomised animals increased their sexual desire as shown by the erection of male genitalia and increased frequency of copulation [Hamilton, 1937; Shapiro, 1937; Stone, 1939; etc.]. It appears therefore, that the production of testosterone in the matured bulls of the present experiment was not influenced by such external factors as air temperature, relative humidity and rainfall.

Volume

From the analysis of variance of total volume of semen it is seen that there was highly significant variation among bulls in their total output of semen per ejaculate. This character, however, was not variable from month to month or from season to season. The present result agrees with those reported by Phillips *et al.* [1943], Swanson and Herman [1944], Salisbury [1944] and Anderson [1946] but is contrary to those obtained by Erb *et al.* [1943] and Mercier and Salisbury [1946].

Total volume of semen is mainly dependent on the secretions of the accessory reproductive organs. It is now well-known that these secretions are dependent upon testosterone liberated by the interstitial cells of the testes. The measurement of the total volume of semen, therefore, as pointed out by Bogart and Mayer [1946] reflects the functional capacity of the interstitial cells. Results obtained in this experiment show that there was individual variation in the functional capacity of the interstitial cells of the testes in bulls but it was not influenced by seasons. This observation, therefore, lends further support to the view that proudction of testosterone is independent of the external factors studied in this experiment.

pH of semen

The hydrogenion-concentration has been recognised by several investigators as one of the many characteristics of semen for evaluation of its quality. From analysis of data for individual bull, *p*H of semen did not seem to have any special significance except that it indicated in a general way other qualities such as, sperm concentration, initial motility and total number of spermatozoa. The *p*H value of semen was usually found to lie between 6·22 to 6·34. Higher *p*H value was associated with low values of other semen characteristics and *vice versa.*

During the latter part of the experimental period, the *p*H value in one bull, however, was found not to lie within the range but was 6·8 or above. As mentioned earlier this was due to an infection in its seminal vesicles. Anderson [1945,*a*] also observed that in conditions involving pathology of genital organs the reaction of semen was characteristically alkaline.

Results obtained in this experiment on the effect of season on *p*H are in agreement with those reported by Erb *et al.* [1942], but not with those obtained by Swanson and Herman [1944], Anderson [1945,*b*] and Raps and Cannon [1947]. It may be mentioned here, that there existed a highly significant seasonal variation in sperm concentration. As the sperm concentration of semen in individual bulls was negatively correlated with the *p*H of semen, it was to be expected, therefore, that a seasonal trend in *p*H should also exist. The results, however, did not show any such trend. This might be due to the fact that *p*H was determined in this investigation with B. D. H. capillators by colorimetric method. Admittedly, the values obtained with this method would be less accurate than what they would have been if determined by glass electrode *p*H meter. But it appears that as *p*H is a general indication of semen quality it failed to show any definite seasonal trend.

Initial motility

Initial motility as estimated by direct microscopical examination is dependent upon the activity of spermatozoa as well as the sperm concentration. Variations in sperm concentration will, therefore, reflect on the degree of initial motility. Analysis of the present data showed highly significant variation among bulls and between different years, and significant variation among seasons. The results, on the whole agree with those reported by Erb *et al.* [1948], Sawnson and Herman [1944], Salisbury [1944] and Anderson [1945,*b*] but differ from the findings of Phillips *et al.* [1943] and Mercier and Salisbury [1946] who reported no significant seasonal variation in the initial motility.

Sperm concentration

Determination of this characteristic is essential in order to appraise the fertilizing capacity of the semen sample. Result of analysis of data on sperm concentration showed that it was highly variable among bulls and among seasons. This agrees with the results reported by previous workers except Anderson [1941] and Swanson and Herman [1944].

Estimation of sperm concentration at regular intervals as was done in this experiment, provided a direct indication of the rate of spermatogenesis. Spermatogenesis is controlled by the activity of the follicle stimulating hormone of the anterior pituitary gland. For full manifestation of its activity, however, the secretions of other endocrines are also necessary. Very recently it has been demonstrated that in some farm animals the seasonal variations in fertility and semen characteristics correspond with the seasonal activity of the thyroid gland and that this seasonal effect can be controverted by artificially administering thyroid hormone in summer and one of the depressant thiouracil drugs in winter [Berliner and Warbritton (1937) Bogart and Mayer 1946]. It seems, therefore, that decrease in sperm concentration in summer and autumn may be due to mild hypothyroidism produced due to the prevailing high environmental temperature and relative humidity.

Total number of spermatozoa

Sperm concentration is indicative of spermatogenetic activity but does not give a true evaluation unless the total number of spermatozoa per ejaculate is also considered. The total number of spermatozoa per ejaculate when calculated from data of two successive ejaculates from an individual animal gives rather an accurate picture of semen quality of the animal for the particular period when the semen was collected. Analysis of data for total number of spermatozoa in different bulls showed no seasonal influence on this character. The present result is in disagreement with those reported by Erb *et al.* [1942] and Phillips *et al.* [1943] but is in agreement with that reported by Mercier and Salisbury [1946]. The latter authors, however, expressed doubts about their results because the frequency of semen collection in their study was not rigorously controlled. Consequently, they believed that the variation due to seasons in this respect might have been obscured. They have, therefore, suggested that 'before the question is satisfactorily answered it will be necessary to establish a rigorous schedule for semen collection throughout a year'. In this investigation, a definite schedule throughout the two consecutive years was followed in collecting semen samples, and yet no significant variation among seasons or among months within seasons was observed.

Abnormal spermatozoa

The result obtained in this respect show that bulls did not differ significantly in production of abnormal spermatozoa. Regarding the effect of seasons it was found that summer differed from the rest of the seasons in producing low percentage of abnormality. Highly significant seasonal variations in abnormal spermatozoa of bulls have also been reported by Erb *et al.* [1942], Phillips *et al.* [1943], Swanson and Herman [1944] and Mercier and Salisbury [1946]. During winter and spring more bent and coiled tail sperms were produced, whereas in summer and autumn more tailless ones. Phillips and his collaborators while working with bulls and rams [1943,*a*; and 1943,*b*] reported a high percentage of abnormality in tails during winter. But they did not mention the types of the tail abnormalities observed. They considered that cold weather might have adversely effected the tails after the collections were made. To test the validity of this view a few fresh samples

of bull semen in small test-tubes were put into chipped ice for about half an hour immediately after collection and later on examined for the percentage of 'bent tail' sperms. Samples thus treated did not show any increase in the appearance of bent tails or coiled tails. Mukherjee and Bhattacharya [1949] have shown that these are not true abnormalities but are immature forms of spermatozoa. It seems, therefore, that during winter and spring when the rate of spermatogenesis is high, a considerable number of immature spermatozoa are carried along with the normal spermatozoa in the male reproductive tract before they come to ampullae for final ejectment.

Blood constituents

From Plates IV and V, it is clear that the two blood constituents, *viz.*, haemoglobin and cell volume were inversely related to the changes in air temperature. Higher the temperature the lesser was the haemoglobin and cell volume contents in blood. Manersa *et al.* [1940] also found that haemoglobin index was higher in winter and lower in summer in Philippine cattle. For both haemoglobin and cell volume the result reported herein agrees with that reported by Pal *et al.* [1945] in dairy cows in India.

During summer air temperature is high as compared with body temperature. As soon as high air temperature comes in contact with skin, some heat is absorbed by peripheral blood which subsequently passes through the heat regulating centre. On coming in contact with blood of higher temperature this centre operates to dispose off the extra heat and as a result the red and white blood corpuscles and platelets go to their reserve places, *i.e.*, spleen and liver. The increased blood plasma in the dialated capillaries evaporates off in the form of perspiration and keep the body cool. Secretions of thyroid and adrenal are also inhibited [Kuno, 1935]. During autumn, high environmental temperature with high humidity perhaps prevent the dissipation of the exrta body heat by retarding body evapcration and thus the physiological adjustment is to a large extent upset. In consequence, there is a general derangement of the body functions including the spermatogenesis of the testes and hence the low concentration of spermatozoa during autumn.

Summary

Seasonal variation in 'reaction-time', semen characteristics, haemoglobin and cell volume contents in the blood of six Kumauni hill bulls were studied during two consecutive years.

Highly significant variation was observed among bulls in average 'reaction-time', all attributes of semen studied except in the percentage of abnormal spermatozoa and the two blood constituents.

'Reaction-time' did not show any definite seasonal trend and bore no relationship with sperm production.

Colour and consistency of semen was found to be thin milky in autumn and milky in the other three seasons.

No significant seasonal variation in average semen volume and total number of spermatozoa was observed.

Highly significant variation was observed in the average initial motility between years; the first year being superior to the second year. It also varied significantly among seasons; summer showing significantly lower motility than winter.

The *p*H of semen showed highly significant variation between years and between months within seasons. *p*H during the first year was lower than in the second.

Highly significant variation in sperm concentration was observed among seasons; autumn showing significantly lower concentration than winter and spring.

Among seasons highly significant variation was found in the percentage of abnormal spermatozoa. In summer the percentage of abnormal spermatozoa was less than in other seasons. Some forms of tail abnormalities such as, bent tail and coiled tail were found to be more preponderant in winter and spring. In autumn and summer, tailless forms were slightly more in comparison with the other two seasons.

Haemoglobin and cell volume contents of blood were highly variable among bulls, between years, among seasons and among months within seasons. The two blood constituents were higher in the second year than in the first. Among seasons, spring was found to be the best and autumn the worst for these characteristics.

The quality of semen and the blood of the bulls were significantly superior in spring, *i.e.*, February to April and significantly inferior in autumn, *i.e.*, August to October. Spring was marked with moderate air temperature, lowest humidity and scanty rainfall. Whereas, autumn recorded as high an air temperature as in summer associated with high humidity and rainfall.

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EFFECT OF MUSCULAR EXERCISE ON SEMEN PRODUCTION

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THE importance of regular exercise for breeding bulls is mentioned in all standard books on animal husbandry. Bartlett and Perry [1939] reported that daily exercise lasting for one hour at the rate of $2\frac{1}{2}$ miles per hour increased semen output by 51 per cent. Kelly [1940] observed that 30 minutes exercise at $2\frac{1}{2}$ miles per hour greatly increased the quality of the semen. He found that motility rose from 5 to 45 per cent before exercise to 60 to 100 per cent after exercise. Without exercise motility ceased at 3 to 24 hours, while with exercise it was maintained up to 22 to 39 hours. The report of Hamilton and Symington [1939] showed that daily exercising increased the volume of ejaculate, sperm concentration and motility, while Dunlop [1941] laid great stress on plenty of exercise for producing larger ejaculates containing more sperm of higher activity.

In recent years, increased attention is being paid to the environment as one of the major factors conditioning the physiological reaction of animals. The breed of the animal also is known to play an equally important part. With the higher air temperature and humidity ranges prevailing in this country, it was thought worthwhile to investigate whether the conclusions on the effect of exercise on semen production arrived at by the foreign workers held good in 'toto', or at least in part, under the environmental 'stresses' met here. The immediate object of undertaking these studies was to arrive at some tested standards for exercising the bulls kept for routine use at artificial insemination centres. In the present paper are reported the results of three series of experiments conducted on a lot of 8 Kumauni-hill bulls.

Experimental procedure

Eight Kumauni-hill bulls receiving a uniform and standard diet were divided at random into 2 groups. The treatments were alloted to the 2 groups at random and at the end of a certain period which varied with the experimental series—the treatments were interchanged, following the 'Switch-back' design described by Brandt [1938].

Experiment I. The experiment I was devised to test the effect of exercise *just prior* to collection. The treatments consisted of an hour's exercise and no exercise. The exercise was imparted by leading the bulls individually round a marked path at an average speed of about three miles an hour. Immediately on the completion of the exercise semen was drawn. Four times in a week collections were made from the bulls and on each day of collection, two ejaculates in rapid succession were taken. The treatments were interchanged between groups at each ensuing

collections, thus group 1 which received exercise before collection on the first day, received no exercise at the second day of collection and exercise again on the third day of collection and so on. The experiment lasted for two weeks. Normally, only two collections in a week are made from routine artificial insemination bulls. The decision to have more collections in a week and confine the experiment to only two weeks was taken in order to eliminate effect of season on semen production. Daily interchanging of treatments between groups reduced the chances of long term cumulative effects, if any, of exercise on semen production, so that only the point at issue namely *immediate* effect of exercise on semen production and reaction time, could be studied.

Experiment II. The experiment was planned to test the long term effect of lack of exercise on semen production and reaction time and was conducted on the same bulls as used in experiment I. The treatments as before consisted of exercise for one hour at the rate of three miles an hour and no exercise. As before the 'Switch-back' design was employed. The treatments were interchanged at the end of a period of one month and, unlike the first experiment, per week only one collection with two ejaculates collected in rapid succession was made. The experiment lasted for four months.

Experiment III. This was designed to test two levels of exercise—one hour and two hours daily—on semen production and reaction time. The experimental design adopted was the same as followed for Experiments I and II. The treatments consisted in daily exercising of bulls for one hour and two hours respectively at the usual rate of three miles an hour. Collections were made as in Experiment II. Unlike Experiment II, the treatments were interchanged between groups at the end of two months. The experiment lasted for four months.

Semen characteristics studied

In each experiment the two ejaculates were treated separately and studied for (i) *Colour* and *consistency* (ii) *Volume* (iii) *Initial motility* (iv) *Initial pH.* (v) *Sperm concentration* (vi) *Per cent of abnormal sperm* (vii) *Total number of sperm in the ejaculum.* In addition, records were maintained of the total 'reaction times'.

Results

In all the experiments the procedure given by Brandt [1942] was followed in analysing the data.

Experiment I. The data are summarized in Table I. The figures given in the table are averages of four observations taken while receiving the treatment. (Exercise or no exercise). A preliminary study in which the various sets of observations on semen characteristics of a given animal while receiving exercise were compared with similar sets while receiving no exercise, showed no clear-cut 'trends'. Secondly, bull-wise comparison of the overall averages of the various semen characteristics of the collections taken in the first week with like averages of the second week also showed no obvious 'trends'. The results of the analysis of variance carried out for each semen characteristic and for each ejaculum are given in Table II.

TABLE I

Showing bull-wise average characteristics of semen and reaction times observed in experiment I

First ejaculate

Bull number	Treatment	Volume (c.c.)	Initial motility	Sperm concentration M/C	Per cent of abnormal sperm	Reaction time (seconds)	Total sperm in ejaculate (Millions)
2	Exercise	1·50	3·88	595	8·75	92	767
	No exercise	1·55	3·63	943	5·00	191	1,275
8	Exercise	1·93	3·00	583	6·75	64	1,459
	No exercise	1·05	3·25	648	5·75	112	710
16	Exercise	1·55	3·38	908	8·00	73	1,505
	No exercise	0·46	1·63	230	7·50	103	107
40	Exercise	1·28	1·88	360	7·75	32	604
	No exercise	1·24	2·13	268	7·25	55	344
1	Exercise	0·88	2·75	613	5·25	97	523
	No exercise	1·40	2·75	295	9·00	79	417
18	Exercise	1·31	2·00	310	3·00	133	562
	No exercise	1·85	1·25	227	5·75	19	368
35	Exercise	1·73	2·00	265	6·00	90	525
	No exercise	2·20	3·25	488	6·00	64	1,065
39	Exercise	1·28	2·75	595	8·50	23	786
	No exercise	2·80	3·63	845	5·50	70	2,340
Average	Exercise	1·43	2·71	529	6·75	75·5	841
	No exercise	1·57	2·69	493	6·47	87	828

TABLE I—*contd.*

Showing bull-wise average characteristics of semen and reaction times observed in experiment I

Second ejaculate

Bull number	Treatment	Volume (c.c.)	Initial motility	Sperm concentration M/C	Per cent of abnormal sperm	Reaction time (seconds)	Total sperm in ejaculate (Millions)
2	Exercise	1·58	3·13	703	5·75	167	1,305
	No exercise	1·64	3·00	725	6·50	170	1,187
8	Exercise	1·33	1·50	988	9·50	50	1,241
	No exercise	0·76	1·88	1,208	8·75	80	778
16	Exercise	0·80	1·75	770	8·75	40	470
	No exercise	0·31	1·50	558	8·00	98	1,612
40	Exercise	1·10	2·00	478	6·50	43	510
	No exercise	1·18	1·63	315	13·25	76	346
1	Exercise	1·11	2·88	493	8·75	162	549
	No exercise	1·65	3·50	748	7·25	147	910
18	Exercise	1·43	1·50	195	11·25	48	296
	No exercise	1·80	2·00	342	6·50	33	603
35	Exercise	1·35	1·38	315	7·50	146	650
	No exercise	2·80	3·25	605	10·75	48	1,647
39	Exercise	0·48	1·50	735	5·25	28	390
	No exercise	1·33	2·00	653	6·75	30	838
Average	Exercise	1·15	1·955	585	7·91	85·5	676
	No exercise	1·43	2·345	644	8·47	85	990

TABLE II

Analysis of variance

Source of Variation	Volume			Motility			Sperm concentration			Per cent of abnormal sperm			Reaction time			Total sperm in ejaculate		
	d.f.	M.S.	F.	d.f.	M.S.	F.	d.f.	M.S.	F.	d.f.	M.S.	F.	d.f.	M.S.	F.	d.f.	M.S.	F.
I *Ejaculate*																		
Between treatments	1	0·2957	2·356	1	17847·12	2·10	1	127033·92	..	1	5·7992	1·13	1	16872240·5	..	1	365736297	..
Error	6	0·1255		6	8491·14		5	195212·63		5	5·1171		6	22402002·5		6	1889490615	
II *Ejaculate*																		
Between treatments	1	1·1566	5·026	1	18430·03	2·68	1	177844·88	..	1	57·6856	1·76	1	80361521	4·52	1	2148991240	2·14
Error	6	0·2301		6	6871·53		5	127148·03		5	32·7121		6	17776563		6	1001171897	

From Table I and Table II it will be seen that—(i) *Volume.* In both ejaculates, the bulls in the 'no-exercise' group produced on an average slightly larger ejaculates than the bulls in the 'exercise' group. The overall mean for the former was 1·50 c.c. as compared to 1·29 c.c. of the latter. F. ratios as worked out separately for the two ejaculates were found to be not significant, while in the 'pooled' analysis F. ratio approached five per cent level of significance.

(ii) *Initial motility.* In the first ejaculate, the mean motility rating was slightly higher for the 'exercise' group than the 'no-exercise' group, while it was the other way about in the second ejaculate. The overall means for the 'exercise' and 'no-exercise' groups were 2·33 and 2·51 respectively. The group differences when considered separately and together for the two ejaculates were not statistically significant.

(iii) *Sperm concentration.* As in the case of the initial motility, sperm concentration was higher for the exercised bulls than the non-exercised ones in the first ejaculate and *vice versa* in the second ejaculate. The respective overall means for the exercised and non-exercised bulls were 557·0 M/C and 568·5 M/C. The difference was not statistically significant.

(iv) *Per cent of abnormal sperm.* The percentage of abnormal sperm followed the same trend as initial motility and sperm concentration. In the first ejaculate, the figure was slightly higher for the exercised than the non-exercised bulls, while in the second ejaculate, it was the other way. The overall group means for the exercised and non-exercised bulls were 7·33 per cent and 7·47 per cent respectively. The difference was not statistically significant.

(v) *Reaction time.* Exercised bulls took on an average lesser time to give both the ejaculates than the non-exercised bulls. The overall means for the two groups were 80·5 seconds and 86·0 seconds respectively. The difference was not statistically significant.

(vi) *Total sperm in an ejaculate.* The exercised bulls on an average gave large number of sperms in the first than in the second ejaculate as compared to the non-exercised bulls ; the overall group means for the bulls in the two lots being 758·5 millions and 909·0 millions respectively. The difference was not statistically significant.

Experiment II. The data are summarised in Table III. The figures in the table are averages of all observations in like treatment periods. In statistical analysis, the question arose as how best to utilise the data within a period. In the previous experiment as only one observation per ejaculate (first or second) was available, this did not present much difficulty. In the present experiment, 4 observations per period of one month were available. Cochran, Autrey and Cannon [1941] who have used a short time switch over design in studying the effect of different rations on milk production have used the total milk production during the experimental period as a single observation for analysis. Branton, Bratton and Salisbury [1947] who employed a similar design in studying the nutritive requirements of dairy bulls used in artificial insemination have used the *average* value instead of the total value

TABLE III

Showing bull-wise average characteristics of semen and reaction times observed in Experiment II

First ejaculate

Bull numbers	Treatment	Volume (c.c.)	Initial Motility	Sperm concentration M/C	Percentage of abnormal sperm	Reaction time (seconds)	Total sperm in ejaculate (million)	Initial pH
60	Exercise	0·49	3·63	1,522	4·13	58	765	5·93
	No exercise	0·50	3·31	1,289	4·88	81	627	5·90
61	Exercise	2·34	3·25	633	5·38	93	1,640	6·01
	No exercise	3·70	3·06	494	3·75	88	1,788	5·96
2	Exercise	1·88	4·06	1,046	4·00	77	2,125	5·88
	No exercise	2·18	4·00	1,038	4·38	102	2,242	5·89
25	Exercise	1·49	3·13	460	6·38	43	680	5·94
	No exercise	1·46	3·06	399	8·13	28	678	6·00
63	Exercise	1·43	3·40	976	5·34	54	1,295	5·90
	No exercise	1·04	3·25	725	7·13	88	798	5·93
64	Exercise	1·21	2·69	470	9·13	82	676	6·23
	No exercise	1·43	3·06	454	9·88	71	903	6·01
1	Exercise	1·29	2·06	209	3·88	23	373	6·39
	No exercise	1·63	3·09	515	5·13	115	1,115	6·04
8	Exercise	2·19	3·94	761	5·25	76	1,770	5·49
	No exercise	2·39	4·13	944	4·88	91	2,367	5·81
Average	Exercise	1·54	3·27	760	5·44	63	1,166	6·03
	No exercise	1·79	3·37	732	6·02	83	1,315	5·94

TABLE III—*contd.*

Showing bull-wise average characteristics of semen and reaction times observed in Experiment II

Second ejaculate

Bull numbers	Treatment	Volume (c.c.)	Initial Motility	Sperm concentration M/C	Percentage of abnormal sperm	Reaction time (seconds)	Total sperm in ejaculate (million)	Initial pH
60	Exercise	0·68	3·73	1,439	3·79	98	706	6·04
	No exercise	0·53	2·25	849	4·67	138	442	6·23
61	Exercise	1·54	3·25	741	5·00	68	844	6·15
	No exercise	2·36	2·75	580	4·75	51	1,246	6·05
2	Exercise	1·35	3·58	1,168	3·17	235	1,380	5·90
	No exercise	2·01	3·60	984	4·46	192	2,028	6·12
25	Exercise	1·76	3·13	511	5·00	69	905	5·93
	No exercise	2·25	3·00	570	3·50	35	1,322	6·00
63	Exercise	1·13	2·73	691	6·79	42	689	6·25
	No exercise	0·93	2·81	631	10·38	62	472	6·06
64	Exercise	1·08	3·13	992	9·63	64	1,115	5·96
	No exercise	1·04	3·13	770	11·88	107	775	5·88
1	Exercise	1·89	3·63	801	3·75	44	1,503	5·99
	No exercise	1·74	3·19	800	3·34	142	1,412	5·82
8	Exercise	2·93	4·00	943	5·38	85	2,424	5·94
	No exercise	2·03	3·44	860	6·50	92	1,720	5·96
Average	Exercise	1·54	3·40	910	5·31	88	1,196	6·02
	No exercise	1·61	3·02	756	6·19	102	1,177	6·02

(which in effect amounts to the same thing) of the data grouped by experimental period as a single observation for statistical treatment. It was argued against this procedure that possibly averaging of observations within a period smoothened out actual effects, if any, due to treatment within that period. This would appear to be so when one considered the possibility of treatment effect being shown only towards the end, if the period was fairly short or after the expiry of some time, if the period was a long one. Keeping these points in view, an attempt was made to study the effect of the nature of grouping of observations in the experimental periods on the statistical analysis. Analyses were made by first taking only the *last* observation within an experimental period before change of treatment, then the average of similar last two observations and finally the average of all the four observations within an experimental period. The corresponding results are shown separately for each characteristic in columns A, B and C of Table IV. The relative precision of

TABLE IV

Showing the error M. S. (Experiment II)

Semen characteristics		First ejaculate		Second ejaculate		Pooled average of the 1st and 2nd ejaculates	
		d.f.	M.S.	d.f.	M.S.	d.f.	M.S.
Volume	A	6	13·11	6	12·65	12	12·88
	B	6	10·64	6	8·30	12	9·47
	C	6	5·25	6	8·52	12	6·88
Initial motility	A	6	15·49	6	8·62	12	12·05
	B	6	1·57	6	7·03	12	4·29
	C	6	1·08	6	2·05	12	1·56
Sperm concentration	A	—	—	—	—	—	—
	B	6	2265486	6	1468490	12	1866988
	C	6	540594	6	716676	12	628635
Percent of abnormal sperm.	A	6	158·66	6	19·58	12	89·12
	B	6	85·82	6	43·45	12	64·63
	C	6	34·31	6	23·88	12	29·09
Reaction time	A	—	—	—	—	—	—
	B	6	12515·64	6	73470·54	12	42993·09
	C	6	6829·94	6	25194·58	12	16012·26
Total sperms	A	—	—	—	—	—	—
	B	6	17521452	6	5122021	12	11321736
	C	6	3828567	6	3082961	12	3455764
Initial pH	A	6	0·47	6	1·20	12	0·83
	B	6	0·07	6	0·57	12	0·32
	C	6	0·13	6	0·27	12	0·20

the estimates got with A, B and C, procedures would be given by the magnitude of the respective errors of the mean estimates; the least value corresponding to

the more precise estimate. Since the error of the mean is dependent on the error M. S., the least value of the error M. S., would also correspond to the best estimate. In Table V are shown the 3 groups of error M. S's found for each criterion studied. Their examination show the existence of a clear 'trend'.* Thus the error M. S. values progressively decreased with the increase in the number of observations averaged—the highest value being given by A where a single observation was used and the least by C where all the four observations were utilised. This suggests that when more than one observation within a period is available, it is undesirable to carry out analysis using only a single observation. This will besides, obviate the necessity of adopting the complicated technique of analysis of incomplete observations, without much risk of any wide deviation from exactitude, if the numbers of observations per period are sufficiently numerous. The straight forward course in such cases would appear to be to take all the observations within a period as done by Cochran, Autrey and Cannon [1941] and Branton, Bratton and Salisbury [1947] for getting more precise results. We have adopted this procedure in analysing the data in this and subsequent experiment and the results discussed are on the basis of averages of *all* observations within a period. The analysis of variance conducted is given in Table V. From Tables III and V it will be seen that:

(i) *Volume.* In both ejaculates, bulls in the 'no-exercise' groups produced on an average larger volumes than those in the 'exercise' group. The overall mean for the former was 1·70 c.c. as compared to 1·54 c.c. of the latter. The difference was not statistically significant.

(ii) *Initial motility.* In the first ejaculate, the mean motility rating was slightly higher for the exercised group than the non-exercised one; while it was the other way about in the second ejaculate. The overall means for the exercised and non-exercised animals were 3·33 and 3·19 respectively. The differences between treatments and ejaculates approached the 5 per cent level of significance, while the interaction treatment ejaculates was highly significant.

(iii) *Sperm concentration.* In both ejaculates, the sperm concentration was higher in the exercised than in the non-exercised bulls. The overall means for the exercised and non-exercised lots were 835 and 744 millions per c.c. respectively. The difference approached the 5 per cent level of significance.

(iv) *Percent of abnormal sperm.* The exercised bulls had a lesser percentage of abnormal sperm than the non-exercised bulls in both the ejaculates. The overall respective means were 5·37 per cent and 6·10 per cent. The difference was significant at 5 per cent level.

(v) *Reaction time.* In both ejaculates, on an average the non-exercised bulls took a longer time than the exercised ones for giving the ejaculates, the overall means being 75 and 92 seconds respectively. The difference was not statistically significant.

* In these cases in which observations were incomplete, the M. S. for procedure A has been omitted as involving complications.

TABLE V

Analysis of variance (II Experiment)

Source of variation	Volume			Initial motility			Sperm concentration			Per cent of abnormal sperm			Reaction time			Total sperm in ejaculate			Initial pH		
	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.
Between treatments	1	0·0002	..	1	7·2603	4·63	1	2867188	4·56	1	206·2814	7·08*	1	56811·9143	3·54	1	33489	..	1	1·0414	5·06*
Between ejaculates	1	4·1371	..	1	7·1824	4·58	1	159746	..	1	0·0105	..	1	31619·0633	1·97	1	28729600	8·31	1	0·0876	..
Treatment and Ejaculate	1	5·9220	..	1	33·1949	21·18**	1	1434682	2·28	1	0·0138	..	1	473·8240	..	1	2521744	..	1	0·7310	3·55
Error	12	6·8870	..	12	1·5667	..	12	628635	..	12	29·1006	..	12	16012·2575	..	12	3455764	..	12	0·2055	..

* Significant at 5 per cent level
** Significant at 1 per cent level

TABLE VI

Showing average bull-wise characteristics of semen and reaction times observed in experiment III

		First ejaculate							Second ejaculate						
Bull Numbers	Treatment	Volume (c.c.)	Initial motility	Sperm concentration (M/C)	Percent of abnormal sperm	Reaction time (seconds)	Total sperm in ejaculate (Million)	Initial pH	Volume (c.c.)	Initial motility	Sperm concentration (M/C)	Percent of abnormal sperm	Reaction time (seconds)	Total sperm in ejaculate (Millions)	Initial pH
40	1 hour exercise	0·78	3·78	1204	2·89	125	714	6·08	0·45	2·17	1307	4·00	132·00	637	6·18
	2 hours exercise	0·53	3·38	1461	7·38	64	1021	6·48	0·99	2·20	485	9·00	89·60	284	6·60
25	1 hour exercise	2·10	3·22	581	6·78	20	1264	5·98	2·08	3·33	516	4·67	11·89	1095	5·99
	2 hours exercise	1·68	2·63	280	4·75	12	579	5·90	1·74	3·75	681	3·63	9·25	1177	6·15
61	1 hour exercise	2·66	3·28	609	4·00	69	1458	6·08	2·66	3·06	618	4·22	103·11	1638	6·11
	2 hours exercise	1·50	4·00	1203	4·00	71	1791	6·20	1·38	3·19	1186	5·38	137·00	1654	6·40
2	1 hour exercise	2·08	4·11	1127	3·56	84	2551	5·93	1·96	3·57	964	3·29	171·83	2018	6·09
	2 hours exercise	1·73	4·56	1154	2·63	121	2032	6·13	1·83	3·13	925	5·25	138·33	1644	6·30
63	1 hour exercise	0·95	2·63	1757	15·38	146	1539	6·31	0·90	2·92	1817	12·83	102·17	1744	6·32
	2 hours exercise	1·37	3·28	1136	8·00	78	1841	6·02	1·33	3·28	709	9·78	47·67	902	6·04
8	1 hour exercise	3·30	4·38	1122	6·25	152	3238	6·10	3·11	4·14	1056	5·86	171·83	3238	6·26
	2 hours exercise	1·66	3·78	744	4·78	86	1234	5·99	2·49	4·44	1203	6·00	75·56	2996	5·93
64	1 hour exercise	1·23	3·31	586	7·50	87	704	6·20	0·89	3·75	1186	9·38	85·00	1117	6·33
	2 hours exercise	2·07	3·33	920	10·78	54	2018	6·02	1·54	3·67	994	10·22	40·44	1763	6·04
1	1 hour exercise	1·50	3·19	484	3·88	43	882	6·05	2·06	3·75	916	7·25	58·25	1741	6·09
	2 hours exercise	1·51	3·93	641	3·29	95	932	5·94	1·97	3·92	738	4·83	138·50	1305	6·00
Average	1 hour exercise	1·83	3·49	934	6·28	91	1544	6·09	1·76	3·34	1047·5	6·44	104·5	1653·5	6·17
	2 hours exercise	1·51	3·61	942	5·70	73	1431	6·085	1·66	3·45	865	6·76	85	1466	6·18

TABLE VII

Analysis of variance experiment III

Source of variation	Volume			Initial motility			Sperm concentration			Per cent of abnormal sperm			Reaction time			Total sperm in ejaculate			Initial pH		
	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.	D.F.	M.S.	F.
Between treatments	1	0·6765	1·33	1	0·2209	..	1	120756	..	1	0·2601	..	1	5786·6449	1·96	1	361502	..	1	0·0001	..
Between ejaculates	1	0·0010	..	1	..	..	1	870	..	1	0·8409	..	1	12·4962	..	1	13865	..	1	0·0342	1·93
Treatment x and ejaculate	1	0·8385	1·65	1	0·0006	..	1	145924	..	1	3·3358	..	1	14·4020	..	1	22575	..	1	0·0012	..
Error	12	0·5062	..	12	0·2403	..	12	243757	..	12	9·3656	..	12	2942·5839	..	12	669040	..	12	0·0177	..

(vi) *Total sperm in an ejaculate.* As in the case of the initial motility, the total sperm was higher for the non-exercised group than in the exercised group in the first ejaculate and *vice versa* in the second ejaculate. The overall means for the two treatments were: Exercise=1181 millions and Non-exercise 1246 millions. The difference was not statistically significant. The between ejaculate difference however, was found to be significant at 5 per cent level.

(vii) *Initial* p*H*. The mean *p*H was lower in the non-exercised lot as compared to the exercise lot in the first ejaculate, while there was no difference in the second ejaculate. The overall means for the exercised and non-exercised bulls were 6·02 and 5·98 respectively. The difference was statistically significant at 5 per cent level.

From the foregoing, it would appear that the attributes most likely to be affected due to lack of regular exercise are initial *p*H, per cent of abnormal sperms, sperm concentration and motility. In the first two attributes, the differences are statistically significant, while in the rest, they approach the 5 per cent level of significance.

Experiment III. The data are summarised in Table VI. The figures in the table are averages of all observations within a period. Using the averages of *all* observations within a period, analysis appropriate to 'switch-back' design was undertaken separately for the first and second ejaculates, for each of the attributes under study. As the error mean squares (carrying 6 d.f each) in the two ejaculates turned out to be homogenous (as determined by F. test) in all cases, a pooled analysis over both ejaculates was carried out. The results are given in Table VII. Examination of Tables VI and VII shows:

(i) *Volume.* In both ejaculates on an average one hour exercised animals gave slightly larger ejaculates than the two hour exercised ones, the respective overall means being 1·79 c.c. and 1·58 c.c. The difference was not statistically significant.

(ii) *Initial motility.* The mean motility rating was higher in both ejaculates, for the two hour exercised animals. The overall means for the one hour and two hour exercised groups were 3·41 and 3·53 respectively. The difference was not statistically significant.

(iii) *Sperm concentration.* In the first ejaculate, the average sperm concentration of animals in the 'one hour exercise' group was lower and in the second ejaculate higher than in the 'two hour exercise' lot. The actual overall means were 990 M/C for the one hour and 903 M/C for the two hour groups respectively. The difference was not statistically significant.

(iv) *Per cent of abnormal sperm.* The average percentage of abnormal sperms was higher in the one hour than in the two hour group in the first ejaculate and *vice versa* in the second ejaculate. The overall means of the one hour and two hour exercised groups were 6·36 per cent, 16·23 per cent, respectively. The difference was not statistically significant.

(v) *Reaction time.* In both ejaculates bulls in 'one hour exercise' group on an average took longer time than those in the 'two hour exercise' group. The overall respective means are 97 and 79 seconds. The difference was not statistically significant.

(vi) *Total sperm in an ejaculate.* In both the caculates, bulls in the one hour exercise group gave larger number of sperm than those in the two hour group, the respective overall means being 1598 and 1448 millions respectively. The difference did not approach the 5 per cent level of significance.

(vii) *Initial* p*H*. In both ejaculates, there was hardly any difference in the values of *p*H of the semen of bulls in the two groups.

On the whole, no significant differences in the various attributes of semen quality and reaction time were found between the animals subjected to the two levels of excercise, showing clearly that no material advantage was gained by increasing the rate of exercise from one to two hours daily.

Summary

1. Experiments were undertaken on Kumauni hill bulls to study the effect of immediate, long term (one month) and two levels of exercise (one hour and two hours on semen production. The exercise (when otherwise stated) consisted in leading the bulls on a marked pathway at the rate of 3 miles an hour for approximately an hour.

2. With the exception of semen volume, on significant difference was observed in the semen characteristics and reaction time of bulls receiving and not receiving exercise *just prior* to collection. Bulls receiving no exercise for a period of one month showed significant changes in intial *p*H and percentage of abnormal sperms while changes in sperm concentration and motility rating approached 5 per cent level of significance. There was no significant difference in the quality of semen and reaction times of bulls receiving one hour and two hour daily exercise. It has been concluded that while lack of exercise for long periods is detrimental to sperm production, increase in the daily level of exercise from one to two hours is not likely to materially improve semen production and reaction time.

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SEASONAL VARIATION IN 'REACTION-TIME' AND SEMEN QUALITY OF SHEEP

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(With one text-figure)

THERE is a distinct breeding season when the testes show maximum reproductive activity in the males of wild forms of domesticated animals [Marshal, 1922]. In the domesticated animals, however, probably as a result of changed environmental conditions attending the process of domestication, the males have become continuous breeders. Radulescu [1933] studied spermatogenesis in rams during all months of the year and found continuous spermatogenesis. Roux and Hoffman [1935] observed that rams do not become impotent during any part of the year. Though spermatogenic activity goes on throughout the year, its extent and rate have been found to depend on environmental temperature. Thus, McKenzie and Berliner [1937] at Missouri found that in Shropshire and Hampshire rams high summer temperature induced a decrease in the numbers of normal and an increase in that of abnormal spermatozoa. Green [1940] observed low sperm concentration and rise in the proportion of abnormal spermatozoa from June to August in Shropshire rams at Minnesota. From January to May the semen quality was best in these rams. Gunn *et al.* [1942] observed marked seasonal degeneration in rams in Australia during hot weather and a gradual recovery in cooler season. Phillips *et al.* [1943, *b*] have made similar observations in Hampshire, Karakul, Shropshire and South down rams at Maryland. In Kenya, seasonal variations in the semen of rams have been observed by Anderson [1945]. One singular fact which emerges out of the review of the literature is that climatic conditions influence very greatly the spermatogenic activity of rams and that high environmental temperature decreases the spermatogenesis.

In the present study an attempt has been made to determine the seasonal variations of semen characteristics of indigenous rams. This was considered worthwhile in view of the fact that the climatic conditions in India are very different from those of other countries and also the sheep of this country belong to distinctly different breeds from those on which similar studies have been conducted.

Material and methods

Experimental animals consisted of 9 rams, $2\frac{1}{2}$ to 4 years of age of the type commonly met within Uttar Pradesh. They were selected for good health and sex ability. Before the commencement of the experiment, they were trained to mount an anoestrous ewe and ejaculate semen in the artificial vagina.

Throughout the experimental period the animals were tied separately in well ventilated sheds and were kept under uniform dietary regime. The concentrates supplied consisted of the following ingredients :—

Rape cake	1000	parts	by	weight.
Wheat bran	200	,,	,,	,,
Gram	600	,,	,,	,,
Indian corn	680	,,	,,	,,
Barley crushed	680	,,	,,	,,
Salt	28	,,	,,	,,

To each animal half a pound of the above mixture was given per day along with 8 lb. of green leaves. In addition the animals were allowed free grazing for six hours a day.

Two collections of semen at an interval of 15 minutes were made from each animal every fortnight for two consecutive years. Each semen sample obtained was examined for the following physical characteristics :—

(1) Colour and consistency of semen.
(2) Total volume of semen.
(3) Initial motility of spermatozoa.
(4) *p*H of semen.
(5) Sperm concentration per c.c. of semen.
(6) Total number of spermatozoa per ejaculate.
(7) Percentage of abnormal spermatozoa.

Besides the above semen characteristics, the 'reaction-time', *i.e.*, the time interval between the release of the male near the female and actual moment of ejaculation, of the animals was noted. The time was measured in seconds.

Colour and consistency were examined by the visual appearance of the semen. Volume was measured to the nearest 1/100 c.c. of semen. Initial motility was scored according to the criterion recommended by Erb, Andrews and Hilton [1942]. Hydrogenion concentration of semen samples was determined by B.D.H. capillator by colorimetric method. Sperm concentration was determined by the usual haemacytometer method. Total number of spermatozoa was determined from the data on total volume and the sperm concentration per unit volume of semen. Percentage of abnormal spermatozoa was determined according to the method of Mukherjee and Bhattacharya [1947].

Results

Data showing the average quality of semen produced by the rams in each month are presented in Table I. Examination of the data reveals that there did not exist any relationship between the 'reaction-time' and quality of semen produced by the rams in each month. Colour of the samples during the entire experimental period was creamy. The consistency of the samples was either thin or thick. From the Table it is apparent that sperm concentration and total number of spermatozoa for thin creamy samples varied from 1666 to 2371 millions of supermatozoa per c.c. of semen and 901 to 1838 millions per ejaculate respectively. Thick creamy samples had the sperm concentration from 2429 to 4951 millions per c.c. of semen and total number of spermatozoa per ejaculate from 1359 to 3891 millions. Thin creamy samples had comparatively higher *p*H than thick creamy samples.

TABLE I

Average monthly variation in 'reaction-time' and semen characteristics of rams

	1945-46.											
	J	F	M	A	M	J	J	A	S	O	N	D
Reaction-time (in seconds)	36	27	38	33	22	27	39	47	40	31	30	33
Colour and consistency	Th. C.	Th. C.	Th. C.	Th. C.	Th. C.	T. C.	T. C.	T. C.	T. C.	T. C.	Th. C.	Th. C.
Volume of semen (in ml.)	0·57	0·76	0·76	0·75	0·60	0·64	0·56	0·54	0·47	0·52	0·54	0·56
Initial motility	4·3	4·7	4·8	4·9	4·8	4·8	4·9	4·0	4·3	4·9	4·3	4·8
pH of semen	6·1	6·1	6·2	6·2	6·0	6·4	6·4	6·1	6·1	6·1	6·0	6·0
Sperm concentration (in millions per ml. of semen)	3,751	4,038	3,553	4,951	2,841	2,371	2,025	1,666	1,901	1,909	3,159	3,685
Total number of spermatozoa (in millions)	2,423	3,141	2,857	3,891	2,033	1,579	1,205	901	1,015	1,305	1,710	2,133
Percentage abnormal spermatozoa.	11·5	3·0	2 ·6	3·0	2·4	3·5	9·0	17·1	5·2	2·8	3·9	7·2

	1946-47											
	J	F	M	A	M	J	J	A	S	O	N	D
Reaction-time (in seconds)	23	24	22	20	23	29	32	29	24	18	18	16
Colour and consistency	Th. C.	Th. C.	Th. C.	T. C.	T. C.	T. C.	T. C.	T. C.	T. C.	T. C.	Th. C.	Th. C.
Volume of semen (in ml.)	0·56	0·59	0·61	0·63	0·54	0·68	0·69	0·62	0·57	0·56	0·54	0·67
Initial motility	4·3	4·5	4·7	4·7	4·9	4·9	4·7	5·0	4·9	4·7	4·8	4·5
pH of semen	6·4	6·4	6·3	6·4	6·3	6·3	6·3	6·4	6·5	6·5	6·4	6·4
Sperm concentration (in millions per ml. of semen)	2,593	3,016	3,268	2,158	2,291	2,591	2,204	1,997	1,855	1,906	2,429	3,236
Total number of spermatozoa (in millions)	1,472	1,931	2,171	1,447	1,302	1,740	1,838	1,415	1,130	1,119	1,359	2,233
Percentage abnormal spermatozoa.	7·2	4·7	4·3	7·1	4·1	7·3	3·9	5·0	7·7	6·9	6·8	10·3

T. C.=Thin creamy
Th. C.=Thick creamy

TABLE II

Seasonal variations in average 'reaction-time' and semen characteristics of rams

	Reaction time (in seconds)	Colour and Consistency of semen	Volume of semen (in c.c.)	Initial motility of spermatozoa	pH of semen	Sperm concentration (in millions per c.c. of semen)	Total no. of spermatozoa (in millions per ejaculate)	Percentage of abnormal spermatozoa per ejaculate
Autumn (August to October)	23·4	T. C.	0·55	4·78	6·3	1,872	1,148	7·45
Winter (November to January)	19·1	Th. C.	0·57	4·62	6·2	3,142	1,892	7·81
Spring (February to April)	22·0	Th. C.	0·69	4·78	6·2	3,497	2,573	4·11
Summer (May to July)	27·8	T. C.	0·62	4·89	6·4	2,787	1,617	5·03

T. C.=Thin creamy
Th. C.=Thick creamy

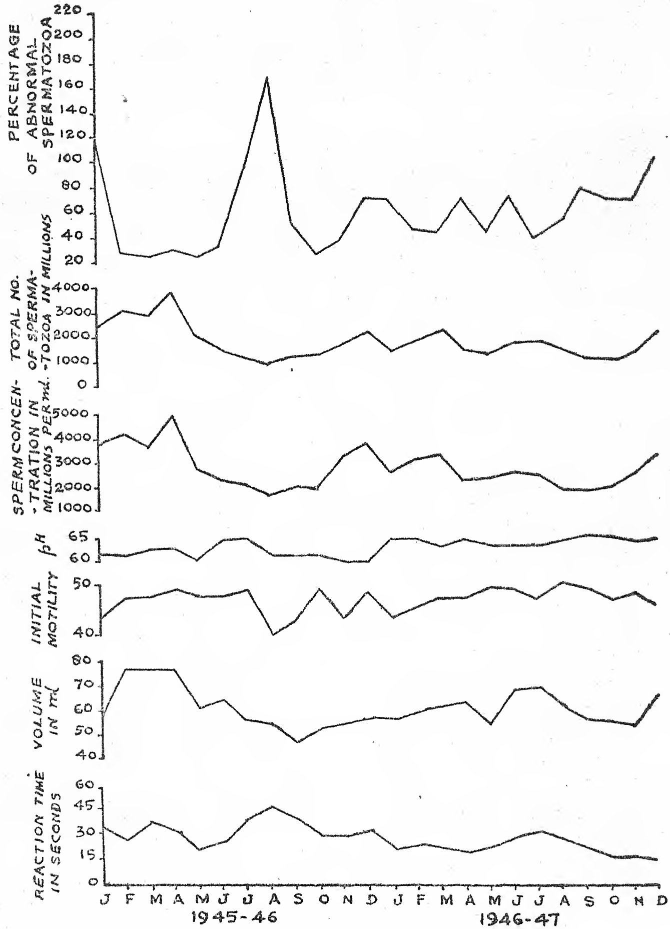

FIG. 1. Showing the month variation.

Average monthly variations in 'reaction-time' and in semen characteristics are presented in fig. I. From the figure it will be seen that the curve for 'reaction-time' did not show any definite monthly variation. Curve for volume showed a tendency to follow those for sperm concentration and total number of spermatozoa. Curves for initial motility and pH did not show wide monthly fluctuations. There is, however, an indication that the average pH was high during the second year than during the first year. The curves for sperm concentration and the total number of spermatozoa are more or less of the same type, showing a definite monthly variation. There was a gradual decrease in both of these characteristics from April to September after which there was a progressive increase. During the second year, as opposed to pH of semen, both of these characteristics were low in comparison with the first year. The curve for abnormal spermatozoa has two peak points in the first year, one in January and the other in August, and in the second year, one in September and the other in December. The high percentage of abnormal spermatozoa in August and September was due to preponderance of tailless spermatozoa, whereas in December and January it was due to bent tail ones.

For the purpose of studying the effect of seasons on 'reaction-time' and semen quality the local seasons were distinguished as follows:

August to October	Autumn
November to January	Winter
February to April	Spring
May to July	Summer

Average 'reaction-time' and characteristics of semen of the rams in different seaons are presented in Table II. Perusal of the table reveals no relationship between the 'reaction-time' and the quality of semen produced in different seasons. There was not much difference in the average 'reaction-time' between autumn and spring; but judged by all the attributes of semen studied, the quality of semen in autumn was decidedly inferior to that in spring. Percentage of abnormal spermatozoa in autumn and winter was comparatively higher than in other two seasons. This was because in autumn and winter large numbers of tailless and bent-tail spermatozoa respectively were found in the ejaculates.

Mukherjee and Bhattacharya [1947] who studied the seasonal variations in semen and blood qualities of bulls during the same period, when this experiment was conducted, found that relative humidity and rainfall were highest in autumn and lowest in spring. Average air temperature in autumn was almost as high as in summer. Air temperature in spring was moderate. It seems, therefore, that when air temperature was moderate with scanty rainfall and lowest humidity as in spring the semen quality of rams improved. Whereas, in autumn when the air temperature was very high with high humidity and rainfall the semen quality deteriorated.

The data on 'reaction-time' and on semen characteristics were subjected to analysis of variance. The summary of the results of analysis are presented in Table III. The table shows that in 'reaction-time' and in all characteristics of semen studied (except in initial motility of spermatozoa) there was highly significant

TABLE III

Summary of the result of analysis of data on 'reaction-time' and semen characteristics of rams

Factors for analysis	Source of variation				
	Between Animals	Between Years	Between seasons	Interaction between seasons and years	Between months within seasons
Reaction-time	**	**	°	°	°
Volume of semen	**	°	**	**	°
Initial motility of spermatozoa	°	°	**	**	**
*p*H of semen	**	**	**	**	**
Sperm concentration	**	**	**	**	**
Total number of spermatozoa	**	**	**	**	**
Percentage of abnormal spermatozoa per ejaculate.	**	°	**	**	**

** Significant at one per cent level.
° Not significant.

variation among rams. Between the two experimental years 1945-46 and 1946-47, the variations observed in 'reaction-time', *p*H of semen, sperm concentration and total number of spermatozoa were highly significant, whereas the variations observed in volume of semen, initial motility and percentage of abnormal spermatozoa were due to chance and not real. Variations between seasons and due to interaction between seasons and years were highly significant in all the characteristics of semen. Between months within seasons highly significant variations were found in initial motility, sperm concentration, total number of spermatozoa and percentage of abnormal spermatozoa.

To test the differences between rams, between years and between seasons with regard to the variable semen characteristics as presented in Table III, critical difference (C.D.) at five per cent level of the variable characteristics have been worked out and are presented in Table IV to VI. The average figure shown under the same bar in these tables did not vary at five per cent level.

Table IV

Critical difference at 5 per cent level of probability in 'reaction-time' and variable semen characteristics among rams

Variable characteristics	Value of C. D.	Averages of the rams are arranged in ascending order.								
Reaction-time	11·0	47 (11·1)	30 (17·3)	46 (18·9)	49 (20·9)	74 (25·6)	65 (36·3)	3 (38·1)	67 (38·6)	27 (55·6)
Volume of semen	0·12	30 (0·37)	46 (0·52)	49 (0·54)	3 (0·55)	65 (0·63)	67 (0·63)	74 (0·72)	47 (0·75)	27 (0·82)
*p*H of semen	0·08	47 (6·08)	27 (6·20)	49 (6·21)	65 (6·26)	46 (6·29)	3 (6·32)	67 (6·34)	74 (6·41)	30 (6·42)
Sperm concentration	503	30 (1,795)	67 (1,880)	74 (1,971)	3 (2,120)	65 (2,417)	46 (2,472)	27 (2,747)	49 (2,895)	47 (3,782)
Total number of spermatozoa	613	30 (667)	3 (1,182)	67 (1,190)	46 (1,345)	74 (1,468)	49 (1,559)	65 (1,764)	27 (2,229)	47 (3,074)
Percentage of abnormal spermatozoa.	2·4	65 (4·6)	67 (4·8)	47 (5·0)	74 (5·1)	27 (5·4)	46 (6·6)	30 (6·8)	49 (6·9)	3 (9·0)

Table V

Critical difference at five per cent level of probability between years

Variable characteristics	Value of C. D. at 5 per cent level	The averages of the years are arranged in ascending order	
Reaction-time	3·5	1946-47 (22·7)	1945-46 (35·6)
*p*H of semen	0·03	1945-46 (6·2)	1946-47 (6·4)
Sperm concentration	237	1946-47 (2,462)	1945-46 (2,987)
Total number of spermatozoa	289	1946-47 (1,599)	1945-46 (2,016)

TABLE VI

Critical difference of variable semen characteristics at 5 per cent level of probability between seasons

Variable characteristics of semen	Value of C. D.	Averages of the seasons are arranged in ascending order			
Volume of semen	0·07	Autumn (0·55)	Winter (0·57)	Summer (0·62)	Spring (0·69)
Initial motility	0·09	Winter (4·62)	Autumn (4·65)	Spring (4·78)	Summer (4·89)
pH of semen	0·04	Winter (6·2)	Spring (6·2)	Autumn (6·3)	Summer (6·4)
Sperm concentration	335	Autumn (1,872)	Summer (2,787)	Winter (3,142)	Spring (3,497)
Total number of spermatozoa	408	Autumn (1,148)	Summer (1,617)	Winter (1,892)	Spring (2,573)
Percentage of abnormal spermatozoa	1·58	Spring (4·11)	Summer (5·03)	Autumn (7·45)	Winter (7·81)

Table IV shows C.D. at five per cent level of the variable characteristics between rams. This table further shows that the 'reaction-time' of rams did not bear any relationship with the quality of semen produced by them. The quality of semen of rams under the same bar which did not show significant variation in their average 'reaction-time' varied considerably. For instance, the quality of semen (as judged by sperm concentration and total number of spermatozoa) of ram 47, whose 'reaction-time' did not vary significantly from rams 30, 46 and 49, was significantly superior to that of these animals. Ram 74 had the average 'reaction-time' significantly less than rams 3 and 67, but the average sperm concentration and total number of spermatozoa of these rams did not vary significantly. Ram 27, whose average 'reaction-time' was significantly more than that of the rest of the rams, had average sperm concentration and total number of spermatozoa lower than only ram 47.

Volume of semen of ram 30 was lower than of all other rams. Rams 46, 49 and 3 had significantly lower semen volume than rams 74, 47 and 27. Rams 65 and 67 had lower semen volume than rams 47 and 27. The rest of the rams did not vary significantly among themselves.

Average pH of semen of ram 47 was significantly lower than of the rest of the animals. It is also evident from the table that the average sperm concentration and total number of spermatozoa of ram 47 were significantly higher than those of the rest of the rams. Semen of ram 27 had significantly lower pH than of rams 46, 3, 67, 74 and 30. Its average sperm concentration was higher than of these animals

except ram 46 with which it did not differ significantly. Similarly ram 49, which had significantly lower *p*H than rams 3, 67, 74 and 30, had significantly higher average sperm concentration than all these rams. It is apparent, therefore, that low *p*H of semen in rams was associated with higher sperm concentration and *vice versa*.

As has been stated before, total number of spermatozoa per ejaculate was obtained by multiplying total volume of semen and the sperm concentration per unit volume. It was expected, therefore, that in rams with higher total volume and sperm concentration the total number of spermatozoa will be higher. This was found to be true in the case of ram 47, which had a higher volume of semen than all other rams excepting ram 27 and also had the highest sperm concentration and total number of spermatozoa. Rams 27, 47 and 74 did not significantly vary in their total volume of semen. But as there was significant variation in their sperm concentration their total number of spermatozoa also significantly varied. Rams 46, 49 and 65 did not significantly vary in their total number of spermatozoa. This was because there was neither significant variation in their total volume of semen nor in their sperm concentration. Rams 30, 3 and 67 did not vary in their total number of spermatozoa although there was significant variation in semen volume between ram 30 and the other two. This might be due to small range of variation between these rams in their sperm concentration. As regards the percentage of abnormal spermatozoa the table shows that ram 3 differed significantly from the rest of the animals.

Critical difference of variable factors between years is presented in Table V. From the table it is apparent that during the second year the rams took significantly lesser time to mount an anoestrous ewe as shown by the 'reaction-time'. The quality of semen during the second year deteriorated as is evident from higher *p*H, lower sperm concentration and the total number of spermatzoa than in the first year. The decrease in the average 'reaction-time' in the second year might be due to the fact that the trained rams after a few months of semen collection at a particular place became so familiar with the place that as soon as they were brought to the place and near an ewe they anticipated service and therefore took lesser time to ejaculate semen in artificial vagina than in the initial stage of the experiment. No satisfactory explanation for the deterioration in semen quality during the second year of the experiment seems possible unless the various contributory factors for semen production are studied in detail, Mukherjee and Bhattacharya [1947] also observed deterioration in semen quality of bulls as shown by higher *pH* and lower initial motility in the second year of their experiment.

Critical difference of the variable semen characteristics between seasons has been presented in Table VI. From the table it is evident that the average volume of semen in autumn and winter was significantly lower than in spring. Initial motility in winter and autumn was lower than in spring and summer. Average *p*H of semen during winter and spring was significantly lower than in autumn and summer. In autumn sperm concentration and total number of spermatozoa were lower than in other seasons. In autumn and winter the percentage of abnormal spermatozoa was higher than in spring and summer. This was due to the appearance of increased numbers of tailless spermatozoa in autumn and 'bent tail' spermatozoa in winter in the ejaculates. From the table it is evident that the semen quality

in rams, judged by pH, sperm concentration and the total number of spermatozoa, deteriorated in autumn and improved in spring.

Discussion

Reaction-time. The study of 'reaction-time' was undertaken with a view to determine if there was any correlation between sex vigour of the animal and its fertility. Result obtained in this experiment showed that sex vigour and sperm production were quite independent of each other. From the records of individual rams it has been found that rams with high sex vigour were not necessarily good semen producers. Further, it has been found that seasons did not greatly influence the sexual behaviour of rams as they did the sperm production. These results agree with those of McKenzie and Berliner [1937] with rams, Anderson [1939] and Mukherjee and Bhattacharya [1947] with bulls.

Volume. Volume of semen was found to be variable among rams and during the seasons. Phillips *et al.* [1943, b] also found significant variation among rams but they did not find significant variation between seasons. McKenzie and Berliner [1937] observed that volume of semen in rams decreased during the periods of high environmental temperature. This observation is in agreement with the results obtained in the present experiment. Under the same climatic conditions in which experiment was performed, Mukherjee and Bhattacharya [1947] observed no such seasonal variation in volume of bull semen. It is apparent, therefore, that the decrease in semen volume in rams during the periods of high temperature associated with high humidity and rainfall might be due to the reason that rams are more susceptible to climatic stress than the bulls.

Motility. Motility rating was determined in this experiment by direct microscopical examination. Obviously this method of estimation not only depends on motility of spermatozoa but on sperm concentration as well. This might be the reason why in autumn when the sperm concentration was lower than in spring the initial motility was also found to be lower. There was no direct proportional increase in initial motility with the increase in sperm concentration during the different seasons. Had it been so, in winter and autumn when there was no significant variation in initial motility there should have been no variation in sperm concentration as well. But the result indicated that in autumn sperm concentration was significantly lower than in the rest of the seasons. Again in summer initial motility was significantly higher than in spring but sperm concentration showed just the reverse trend. Initial motility rating as has been done in this experiment is the most emperical method of evaluating semen quality. At best it can indicate a good sample from a bad sample. Grading of fairly good samples by this method alone is almost impossible. This might be the reason why, although there was highly significant variation in sperm concentration among rams and between years, initial motility failed to show any significant variation either among rams or between years. Phillips *et al.* [1943, b] obtained significant variation between rams within breed as well as between seasons which is in disagreement with the result of the present investigation. Our result is, however, in agreement with what has been reported by Phillips and his co-workers [1943, a] in bulls.

Hyderogenion concentration. From the records of individual animals it has been found that semen samples with higher pH value were usually poorer

in sperm concentration. McKenzie and Berliner [1937] also found that the normal ejaculates of rams with sperm concentration higher than 1000 millions per c.c. were usually acidic and sometimes gave a value as low as 5·9 and never above 7·3. Comstock and Brady [1937] gave the *p*H of normal semen of ram as 6·9 and abnormal as over 7·0, Terril [1937] observed that ejaculates giving an acid reaction to litmus were definitely superior to those giving an alkaline reaction. In the present investigation, although it has been found that rams with higher *p*H of semen had lower sperm concentration, the correlation was not directly proportional. Determination of *p*H in semen has been recognised by various workers on semenology as one of the methods of evaluating semen quality as it indirectly gives an indication of sperm concentration in semen. Higher *p*H of semen in generally associated with lower sperm concentration and *vice versa*. This was found to be true between years and between seasons. In autumn and summer when the air temperature was higher than in winter and spring the *p*H value of semen was higher associated with lower sperm concentration. This observation is confirmatory to the results obtained by McKenzie and Berliner [1937] in rams under heat treatment. They observed that semen in rams under heat treatment became more and more alkaline. Gunn and his co-workers [1942] also observed that semen of rams whose tests were subject to scrotal insulation always became alkaline.

Sperm concentration. Result of analysis of data on sperm concentration showed that it was highly variable among rams between years and among seasons. Highly significant variation between rams within breeds and between seasons have also been observed by Phillips *et al.* [1943]. They found the highest sperm concentration in winter and lowest in fall. McKenzie and Berliner [1937] observed a low level of sperm concentration in the Shorpshire during August and September and a sudden increase in October, which lasted till December. In this experiment highest sperm concentration was observed in spring and lowest in autumn. This result is in complete agreement with that obtained in bulls by Mukherjee and Bhattacharya [1947]. Berliner and Werbritton [1937] made histological studies of the organs and glands of the rams which were used in the experiment of McKenzie and Berliner [1937]. They also made histological studies of organs and glands of thyroidectomised rams and rams treated with thyroxine and gonadotrophins. Their results indicated that the change in level of thyroid secretion due to high environmental temperature might be the cause of lowered fertility in rams. Bogart and Mayer [1946] found that thyroid is of major importance in the reproductive physiology of the rams and that environmental temperatures produce variations in rams indirectly through the thyroid gland. High environmental temperatures produce variations in rams indirectly through the thyroid gland. High environmental temperature decrease thyroid secretion and consequently decrease the spermatogenesis.

Total number of spermatozoa. The result of analysis of the data on total number of spermatozoa showed that autumn was the worst season and spring the best season for total number of spermatozoa. In winter and summer there was no significant variation. This result differs from the observation of McKenzie and Berliner [1937] and Phillips *et al.* [1943, b]. According to McKenzie

and Berliner a distinct increase in total number of spermatozoa was observed from October to January in Shorpshire and August to November in Hampshire. According to Phillips and his associates, total spermatozoa was highest in winter and lowest in fall. Both the above groups of workers found spring to be the second best season.

Abnormal spermatozoa. The result obtained in this respect show that ram 3 varied significantly from the rest of the rams. Regarding the effect of seasons, it was found that in autumn and spring the average percentage of abnormal spermatozoa was significantly more than in spring and summer. It has been stated before that in autumn the tailless spermatozoa and in winter the bent-tail ones were more than in other seasons. Autumn was marked for its high temperature, humidity and rainfall. During this season the semen quality was also poorer than in other seasons. Phillips and McKenzie [1934] observed that there was a marked increase in the production of abnormal spermatozoa following heat treatment of rams and within three weeks practically no spermatozoa were present. McKenzie and Berliner [1937] also found that heat applied to scrotum either experimentally or by local inflammation decreased spermatogenesis and increased abnormal spermatozoa. The predominance of tailless spermatozoa in autumn as found in this experiment might be due to high environmental temperature. The increase in the percentage of tail abnormalities in winter as observed in this experiment was also reported in bulls and rams by Phillips *et al.* [1943, a and 1943 b]. But they did not mention the types of the tail abnormalities. This experiment showed that the predominant tail abnormality in winter was the bent-tail one. Mukherjee and Bhattacharya [1947] also observed the increase in bent-tail spermatozoa in the ejaculates of bulls in winter. They have further shown that the bent-tail spermatozoa in the ejaculates of farm animals are really immature ones and have suggested that in winter, when the rate of spermatogenesis was comparatively higher than in autumn, more of immature spermatozoa like bent-tail ones were carried along with normal spermatozoa in the male reproductive tract before they came to ampullae for final ejectment.

Summary

Seasonal variation in 'reaction-time' and semen characteristics of nine rams were studied for two consecutive years.

Highly significant variation was observed among rams in average 'reaction-time' and all attributes of semen studied except in the initial motility.

'Reaction-time' did not show any definite seasonal trend and bore no relationship with the quality of semen produced.

Between years highly significant variations were found in 'reaction-time', pH of semen, sperm concentration and total number of spermatozoa.

Between seasons highly significant variations were found in all characterisitcs of semen except in 'reaction-time'. The semen quality in autumn was poorer than in other seasons. Spring was found to be the best season for semen quality.

Between months within seasons highly sigificant variation was found in intial motility of spermatozoa, sperm concentration, total number of spermatozoa and percentage of abnormal spermatozoa.

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COMPOSITION OF MILK OF INDIAN ANIMALS

III.—FREEZING POINT, LACTOSE AND CHLORIDE CONTENT OF MILK SAMPLES FROM DIFFERENT FARMS IN INDIA

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IN a previous communication [Dharmarajan, Venkateswara Rao, Menon and Dastur, 1950], results of analysis of nearly 1,300 samples of milk produced at the Indian Dairy Research Institute, Bangalore, from herd and individual animals were discussed. It was shown that the average value for the freezing point of cow and buffalo milk was identical, *viz.*—0·548°C. Over 96 per cent of the samples studied showed a freezing point depression greater than 0·530°C. and hence this value was recommended for use for legal purposes. Buffalo milk was found to be richer in lactose content than cow milk. The average value for lactose content of cow milk was 4·92 per cent, and of buffalo milk 5·26 per cent. On the other hand, cow milk contained a larger amount of chlorine than buffalo milk, the average values being 0·089 and 0·072 per cent, respectively.

It was thought desirable to extend the study and compare the results with those of milk samples collected from other farms in India. All the samples were obtained from dairy farms in different parts of the country where animals were milked under strict supervision.

EXPERIMENTAL

For the collection of samples to be obtained from long distances a preservative had to be used. From a large number of trails it was found that mercuric perchloride in the concentration of 0·075 per cent was the most suitable preservative [Venkateswara Rao, Dastur and Dharmarajan, 1950]. The avarage increase in freezing point depression by the addition of mercuric perchloride in this concentration was 0·02°C.

Samples were obtained in 300 ml. glass bottles closed with rubber stoppers. It was previously ascertained that rubber stoppers had no effect on the freezing point. A 4·5 per cent solution of mercuric perchloride in ethyl ether was prepared and 5 ml. of this added to each sample bottle. The ether was then removed under suction. In this way the addition of extraneous water was avoided. These bottles were despatched to the respective collecting centres with instructions to fill them up to the mark and send the samples as early as possible to Bangalore by post. A record was kept of the breed of animals from which samples were obtained, the number of animals in the herd and the number of days after which the sample was analysed in the laboratory.

Freezing point, lactose and chlorine were determined by the methods already described in previous communication [Dharmarajan, *et al.*, 1950]. The apparent

values for freezing point obtained were corrected by subtracting 0·02°C. from the observed values. Similarly, chlorine percentage found was corrected by the amount contributed by the added preservative. In Tables I and II, only corrected figures are given. The average data for milk of different breeds of animals are summarised in Table III.

TABLE I

Freezing point, lactose and chlorine in cow milk samples from different farms in India

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose monohydrate per cent	Chlorine per cent
1	Agra	Crossbred	3	8	0·545	5·07	0·087
2	,,	,,	4	10	0·560	5·06	0·095
3	,,	,,	4	9	0·536	4·55	0·122
4	Allahabad	,,	45	10	0·538	5·20	0·072
5	,,	,,	70	9	0·539	5·22	0·072
6	,,	,,	60	6	0·530	5·24	0·064
7	,,	,,	56	5	0·544	5·22	0·068
8	Ambala	,,	85	15	0·540	5·20	0·088
9	,,	,,	87	7	0·530	5·35	0·082
10	,,	,,	90	9	0·530	5·07	0·081
11	Bombay	,,	6	6	0·547	4·62	0·110
12	,,	,,	147	5	0·540	..	..
13	,,	,,	245	5	0·533	5·68	0·060
14	,,	,,	258	16	0·534	5·60	0·063
15	,,	,,	260	15	0·543	5·34	0·060
16	,,	,,	278	12	0·536	5·68	0·050
17	,,	,,	349	9	0·530	5·38	0·060
18	,,	,,	358	6	0·542	5·36	0·065
19	Dalhousee	,,	44	8	0·573	5·06	0·094
20	,,	,,	36	9	0·550	5·60	0·091
21	,,	,,	51	13	0·550	4·85	0·093
22	,,	,,	54	12	0·530	4·33	0·091
23	,,	,,	50	11	0·555	..	..
24	,,	,,	45	9	0·542	4·70	0·089
25	Jullundar	,,	8	7	0·548	4·81	0·122
26	,,	,,	9	7	0·555	5·51	0·065
27	,,	,,	16	7	0·556	5·49	0·070
28	,,	,,	7	7	0·558	5·21	0·084
29	,,	,,	13	7	0·544	5·65	0·052
30	,,	,,	9	7	0·550	4·76	0·114
31	,,	,,	22	10	0·563	..	..

TABLE I—*contd.*

Freezing point, lactose and chlorine in cow milk samples from different farms in India—contd.

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose monohydrate per cent	Chlorine per cent
32	Jullundar	Crossbred	9	10	0·553	..	..
33	,,	,,	8	10	0·550	..	..
34	Kasauli	,,	1	7	0·560	5·34	0·076
35	,,	,,	1	6	0·553	4·89	0·101
36	,,	,,	1	7	0·540	4·48	0·103
37	Lahore	,,	2	24	0·550	4·78	0·123
38	,,	,,	21	24	0·551	5·38	0·085
39	,,	,,	4	24	0·545	5·23	0·088
40	,,	,,	8	24	0·531	5·21	0·088
41	,,	,,	3	24	0·525	5·10	0·075
42	Mysore	,,	3	3	0·530	4·71	0·121
43	,,	,,	1	3	0·540	5·53	0·074
44	,,	,,	1	3	0·546	5·34	0·115
45	Sialkot	,,	24	11	0·545	4·78	0·097
46	,,	,,	18	7	0·550	5·88	0·081
47	,,	,,	5	8	0·540	5·87	0·090
48	,,	,,	9	8	0·545	5·22	0·069
49	,,	,,	19	8	0·552	5·21	0·079
50	,,	,,	3	12	0·550	5·36	0·076
51	,,	,,	8	10	0·540	5·22	0·074
	Average				0·544	5·14	0·084
52	Wardha	Gaolao	12	16	0·548	5·52	0·058
53	,,	,,	13	15	0·544	5·53	0·054
54	,,	,,	14	6	0·540	5·45	0·061
55	,,	,,	10	10	0·570	5·44	0·059
	Average				0·551	5·49	0·058
56	Bombay	Gir	12	7	0·544	5·51	0·075
57	,,	,,	198	3	0·550	..	..
58	,,	,,	199	2	0·531	4·46	0·104
59	,,	,,	234	6	0·535	4·76	0·109
60	,,	,,	271	14	0·535	5·22	0·076
61	,,	,,	400	6	0·550	5·51	0·050
	Average				0·541	5·09	0·083
62	Hosur	Hallikar	..	4	0·574	5·18	0·082
63	,,	,,	2	4	0·545	5·67	0·058
64	,,	,,	2	4	0·540	5·38	0·069

TABLE I—*contd.*

Freezing point, lactose and chlorine in cow milk samples from different farms in India—contd.

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose monohydrate per cent	Chlorine per cent
65	Hosur	Hallikar	15	3	0·539	5·22	0·080
66	,,	,,	17	4	0·536	5·53	[illegible]
67	,,	,,	11	4	0·552	5·98	0·043
68	,,	,,	17	4	0·540	5·59	0·055
69	,,	,,	14	4	0·548	5·53	0·055
70	,,	,,	10	2	0·542	..	..
71	,,	,,	8	2	0·540	5·56	0·060
72	,,	,,	12	11	0·570	5·52	0·064
73	,,	,,	16	4	0·540	5·37	0·066
74	,,	,,	9	5	0·560	..	..
75	,,	,,	7	8	0·545	5·53	0·063
76	,,	,,	9	7	0·552	5·37	0·067
77	,,	,,	10	3	0·540	5·37	0·064
78	,,	,,	11	8	0·550	5·53	0·061
79	,,	,,	7	4	0·550	5·68	0·054
80	,,	,,	11	4	0·548	5·82	0·057
81	,,	,,	..	2	0·575	5·83	0·061
82	Mysore	,,	4	3	0·535	5·31	0·054
83	,,	,,	1	3	0·548	5·66	0·063
	Average				0·549	5·53	0·062
84	Dacca	Hariana	10	9	0·533	5·51	0·062
85	,,	,,	5	10	0·547	5·51	0·062
86	Lucknow	,,	16	20	0·547	4·73	0·095
87	Agra	,,	3	8	0·545	5·07	0·087
88	,,	,,	4	10	0·560	5·06	0·095
	Average				0·546	5·18	0·080
89	Hissar	Hissar	30	9	0·530	5·21	0·072
90	,,	,,	50	7	0·555	5·11	0·080
	Average				0·543	5·16	0·076
91	Hosur	Kangayam	..	4	0·560	5·35	0·073
92	,,	,,	20	4	0·538	5·36	0·057
93	,,	,,	25	4	0·528	5·38	0·055
94	,,	,,	24	3	0·530	5·46	0·063
95	,,	,,	25	4	0·542	5·82	0·057
96	,,	,,	25	4	0·542	5·32	0·055
97	,,	,,	29	4	0·530	5·29	0·059

TABLE I—*contd.*

Freezing point, lactose and chlorine in cow milk samples from different farms in India—contd.

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose monohydrate per cent	Chlorine per cent
98	Hosur	Kangayam	22	4	0·545	5·38	·064
99	,,	,,	29	2	0·530	5·07	0·073
100	,,	,,	27	11	0·565	5·37	0·068
101	,,	,,	31	4	0·540	5·37	0·069
102	,,	,,	31	5	0·545	..	..
103	,,	,,	29	8	0·546	5·35	0·068
104	,,	,,	25	7	0·543	5·37	0·064
105	,,	,,	21	5	0·540	5·52	0·078
106	,,	,,	26	5	0·550	5·53	0·059
107	,,	,,	18	4	0·542	5·51	0·063
108	,,	,,	..	2	0·578	5·52	0·068
	Average				0·544	5·41	0·064
109	Bombay	Kathiawadi*	21	5	0·543	5·36	0·077
110	,,	,,	99	7	0·542	5·53	0·049
111	,,	,,	134	6	0·532	5·68	0·057
112	,,	,,	204	6	0·545	4·90	0·110
113	,,	,,	209	5	0·545	4·91	0·094
114	,,	,,	215	6	0·540	..	..
115	,,	,,	246	5	0·532	..	..
116	,,	,,	320	9	0·545	5·66	0·089
117	,,	,,	323	7	0·544	5·53	0·084
118	,,	,,	339	8	0·530	5·08	0·076
119	,,	,,	345	6	0·535	5·67	0·058
120	,,	,,	353	7	0·550	5·51	0·043
121	,,	,,	359	8	0·545	..	..
122	,,	,,	369	6	0·535	..	..
123	,,	,,	363	7	0·530	..	..
124	,,	,,	367	6	0·535	..	..
125	,,	,,	365	5	0·535	..	..
126	,,	,,	378	10	0·540	5·36	0·076
127	,,	,,	388	7	0·540	5·36	0·082
128	,,	,,	392	6	0·541	5·36	0·080
129	,,	,,	394	5	0·535	5·52	0·089
130	,,	,,	401	5	0·542	5·67	0·065
131	,,	,,	387	8	0·555	5·66	0·067
	Average				0·542	5·42	0·068

(*Samples were received under this designation though there is no officially recognised breed of this name.)

TABLE I—*contd.*

Freezing point, lactose and chlorine in cow milk samples from different farms in India—contd.

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing Point —°C.	Lactose monohydrate per cent	Chlorine per cent
132	Delhi	Sahiwal	49	5	0·536	5·56	0·068
133	,,	,,	53	5	0·533	5·51	0·067
134	,,	,,	58	9	0·542	5·36	0·066
135	,,	,,	63	10	0·530	5·28	0·068
136	,,	,,	67	17	0·544	5·39	0·055
137	,,	,,	75	6	0·540	5·52	0·068
138	,,	,,	73	8	0·550	5·05	0·064
139	,,	,,	70	10	0·554	4·62	0·063
140	,,	,,	71	11	0·555	4·99	0·066
141	Jallundar	,,	1	7	0·561	5·50	0·141
142	,,	,,	1	10	0·575	..	..
143	Ferozepore	,,	12	6	0·528	5·21	0·068
144	Telankheri, M.P.	,,	24	4	0·541	5·07	0·103
145	,,	,,	24	3	0·543	5·07	0·083
146	,,	,,	31	7	0·565	4·88	0·080
147	Koradih, M.P.	,,	19	29	0·535	..	..
148	,,	,,	18	21	0·542	5·06	0·078
149	,,	,,	17	20	0·553	5·22	0·074
150	,,	,,	..	4	0·553	..	..
	Average				0·545	5·22	0·076
151	Allahabad	Red Sindhi	25	10	0·540	4·94	0·084
152	,,	,,	30	6	0·527	5·24	0·060
153	,,	,,	35	5	0·535	5·22	0·066
154	,,	,,	30	6	0·545	..	..
155	,,	,,	27	6	0·535	5·34	0·064
156	Hosur	,,	..	4	0·554	4·89	0·099
157	,,	,,	40	4	0·535	5·31	0·067
158	,,	,,	38	4	0·530	5·22	0·061
159	,,	,,	35	4	0·537	5·19	0·068
160	,,	,,	37	4	0·540	5·38	0·064
161	,,	,,	36	2	0·506	5·06	0·064
162	,,	,,	43	4	0·563	5·36	0·066
163	,,	,,	43	5	0·535	..	..
164	,,	,,	40	8	0·537	5·22	0·072
165	,,	,,	40	7	0·545	5·36	0·061
166	,,	,,	40	5	0·530	5·36	0·066

TABLE I—*concld.*

Freezing point, lactose and chlorine in cow milk samples from different farms in India—concld.

Sample number	Place of origin	Breed	Number of animal in the herd	Samples analysed after days	Freezing point —°C	Lactose monohydrate per cent	Chlorine per cent
167	Hosur	Red Sindhi	40	5	0·540	5·22	0·064
168	,,	,,	39	4	0·560	5·37	0·060
169	,,	,,	35	4	0·525	5·36	0·065
170	Karachi	,,	About 60	11	0·563	5·06	0·065
171	,,	,,	,,	8	0·540	4·76	0·087
172	,,	,,	,,	9	0·560	5·23	0·065
173	,,	,,	,,	10	0·550	5·66	0·050
174	,,	,,	,,	7	0·570	5·38	0·058
175	,,	,,	,,	9	0·545	5·36	0·059
176	,,	,,	,,	11	0·565	5·06	0·060
177	,,	,,	,,	7	0·554	6·15	0·054
178	Poona	,,	3	8	0·537	5·21	0·074
179	,,	,,	3	7	0·550	5·08	0·094
180	,,	,,	4	6	0·530	5·08	0·081
181	,,	,,	4	7	0·535	4·76	0·073
182	,,	,,	3	6	0·560	5·36	0·070
183	,,	,,	4	7	0·540	4·60	0·096
184	,,	,,	3	6	0·530	..	..
185	,,	,,	4	5	0·546	5·11	0·061
186	,,	,,	3	6	0·552	4·82	0·071
187	,,	,,	4	5	0·561	5·11	0·061
	Average				0·543	5·20	0·068
188	Karavai	Tharparkar	..	11	0·540	5·18	0·050
189	,,	,,	..	9	0·558	5·83	0·050
190	,,	,,	..	11	0·566	5·37	0·078
191	,,	,,	..	7	0·561	5·37	0·065
192	,,	,,	..	9	0·555	5·39	0·060
193	,,	,,	..	11	0·565	5·51	0·061
194	,,	,,	..	15	0·548	4·62	0·063
195	,,	,,	..	7	0·545	5·08	0·082
196	Karnal	,,	20	9	0·528	5·45	0·055
197	,,	,,	20	6	0·550	5·37	0·062
198	,,	,,	14	10	0·550	..	..
199	,,	,,	18	6	0·542	5·51	0·061
200	,,	,,	20	6	0·544	5·28	0·050
201	,,	,,	18	7	0·548	5·20	0·061
202	,,	,,	20	5	0·531	5·49	0·063
	Average				0·549	5·83	0·062
Average for all cow milk samples					0·545	5·27	0·072

TABLE II

Freezing point, lactose and chlorine in buffalo milk samples from different farms in India

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose Monohydrate per cent	Chlorine per cent
1	Allahabad	Murrah	12	10	0·540	4·96	0·062
2	,,	,,	15	9	0·532	5·37	0·056
3	,,	,,	14	5	0·540	5·52	0·049
4	,,	,,	14	8	0·555	5·66	0·052
5	,,	,,	15	10	..	5·65	0·059
6	Ambala	,,	28	15	0·550	5·38	0·059
7	,,	,,	27	7	0·530	5·64	0·062
8	Dacca	,,	50	9	0·545	5·53	0·041
9	,,	,,	100	11	0·540	5·59	0·053
10	Hosur	,,	1	4	0·535	5·62	0·057
11	,,	,,	1	4	0·566	5·88	0·080
12	,,	,,	1	2	0·554	..	..
13	,,	,,	1	2	0·540	5·53	0·058
14	,,	,,	1	4	0·534	5·66	0·079
15	,,	,,	1	4	0·577	5·71	0·064
16	Madras	,,	9	6	0·530	5·34	0·059
17	,,	,,	28	17	0·550	5·54	0·050
18	,,	,,	20	4	0·525	5·51	0·042
19	Mahurzari, M.P.	,,	9	34	0·534	..	..
20	,,	,,	8	14	0·527	5·49	0·066
21	,,	,,	6	6	0·540	5·20	0·077
22	,,	,,	4	5	0·556	5·22	0·071
	Average				0·543	5·50	0·060
23	Mahurzari, M.P.	Nagpuri	22	34	0·530	..	..
24	,,	,,	24	14	0·532	5·64	0·064
25	,,	,,	20	6	0·538	5·34	0·065
26	,,	,,	16	5	0·561	5·20	0·065
	Average				0·542	5·39	0·065
27	Poona	Surti	3	8	0·532	5·35	0·070
28	,,	,,	2	7	0·540	5·81	0·066
29	,,	,,	5	6	0·542	5·66	0·061
30	,,	,,	4	7	0·552	5·50	0·053
31	,,	,,	3	6	0·542	5·81	0·055
32	,,	,,	4	4	0·560	5·50	0·053
33	,,	,,	4	7	0·550	5·34	0·061
34	,,	,,	3	6	0·535	..	..

TABLE II—*contd.*

Freezing point lactose and chlorine in Buffalo milk samples from different farms in India—contd.

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose Monohydrate per cent	Chlorine per cent
35	Poona	Surti	3	5	0·558	5·34	0·064
36	,,	,,	4	6	0·548	5·34	0·050
37	,,	,,	4	5	0·548	4·68	0·063
	Average	..	..	..	0·546	5·43	0·060
Average for all buffalo milk samples			..	..	0·544	5·47	0·060

TABLE III

Summary of results for freezing point, lactose and chlorine of milk samples from different farms in India

Breed	Number of samples examined	Freezing point —°C.	Lactose monohydrate per cent	Chlorine per cent	Lactose chlorine number
		Cow milk samples			
Cross bred	42	0·544	5·14	0·084	6·27
Gaolao	4	0·551	5·49	0·058	6·27
Gir	6	0·541	5·09	0·083	6·21
Hallikar	22	0·549	5·53	0·062	6·37
Hariana	5	0·546	5·18	0·080	6·26
Hissar	2	0·543	5·16	0·076	6·19
Kangayam	18	0·544	5·41	0·064	6·38
Kathiwadi	23	0·542	5·42	0·068	6·34
Sahiwal	19	0·545	5·22	0·076	6·25
Red Sindhi	37	0·543	5·20	0·068	6·12
Tharparkar	15	0·549	5·33	0·061	6·15
Average for all cow milk samples.	202	0·545	5·27	0·072	6·34
		Buffalo milk samples			
Murrah	22	0·543	5·50	0·060	6·31
Nagpuri	4	0·542	5·39	0·065	6·27

Table II

Freezing point, lactose and chlorine in buffalo milk samples from different farms in India

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose Monohydrate per cent	Chlorine per cent
1	Allahabad	Murrah	12	10	0·540	4·96	0·062
2	,,	,,	15	9	0·532	5·37	0·056
3	,,	,,	14	5	0·540	5·52	0·049
4	,,	,,	14	8	0·555	5·66	0·052
5	,,	,,	15	10	..	5·65	0·059
6	Ambala	,,	28	15	0·550	5·38	0·059
7	,,	,,	27	7	0·530	5·64	0·062
8	Dacca	,,	50	9	0·545	5·53	0·044
9	,,	,,	100	11	0·540	5·59	0·053
10	Hosur	,,	1	4	0·535	5·62	0·057
11	,,	,,	1	4	0·566	5·88	0·080
12	,,	,,	1	2	0·554	..	..
13	,,	,,	1	2	0·540	5·53	0·058
14	,,	,,	1	4	0·534	5·66	0·079
15	,,	,,	1	4	0·577	5·71	0·064
16	Madras	,,	9	6	0·530	5·34	0·059
17	,,	,,	28	17	0·550	5·54	0·050
18	,,	,,	20	4	0·525	5·51	0·042
19	Mahurzari, M.P.	,,	9	34	0·534	..	..
20	,,	,,	8	14	0·527	5·49	0·066
21	,,	,,	6	6	0·540	5·20	0·077
22	,,	,,	4	5	0·556	5·22	0·071
	Average				0·543	5·50	0·060
23	Mahurzari, M.P.	Nagpuri	22	34	0·539	..	..
24	,,	,,	24	14	0·532	5·64	0·064
25	,,	,,	20	6	0·538	5·34	0·065
26	,,	,,	16	5	0·561	5·20	0·065
	Average				0·542	5·39	0·065
27	Poona	Surti	3	8	0·532	5·35	0·070
28	,,	,,	2	7	0·540	5·81	0·068
29	,,	,,	5	6	0·542	5·66	0·061
30	,,	,,	4	7	0·552	5·50	0·053
31	,,	,,	3	6	0·542	5·81	0·055
32	,,	,,	4	4	0·560	5·50	0·053
33	,,	,,	4	7	0·550	5·34	0·061
34	,,	,,	3	6	0·535	..	..

TABLE II—*contd.*

Freezing point lactose and chlorine in Buffalo milk samples from different farms in India—contd.

Sample number	Place of origin	Breed	Number of animals in the herd	Samples analysed after days	Freezing point —°C.	Lactose Monohydrate per cent	Chlorine per cent
35	Poona	Surti	3	5	0·558	5·34	0·064
36	,,	,,	4	6	0·548	5·34	0·050
37	,,	,,	4	5	0·548	4·68	0·063
	Average	..	..	..	0·546	5·43	0·060
Average for all buffalo milk samples			..	..	0·544	5·47	0·060

TABLE III

Summary of results for freezing point, lactose and chlorine of milk samples from different farms in India

Breed	Number of samples examined	Freezing point —°C.	Lactose monohydrate per cent	Chlorine per cent	Lactose chlorine number
		Cow milk samples			
Cross bred	42	0·544	5·14	0·084	6·27
Gaolao	4	0·551	5·49	0·058	6·27
Gir	6	0·541	5·09	0·083	6·21
Hallikar	22	0·549	5·53	0·062	6·37
Hariana	5	0·546	5·18	0·080	6·26
Hissar	2	0·543	5·16	0·076	6·19
Kangayam	18	0·544	5·41	0·064	6·38
Kathiwadi	23	0·542	5·42	0·068	6·34
Sahiwal	19	0·545	5·22	0·076	6·25
Red Sindhi	37	0·543	5·20	0·068	6·12
Tharparkar	15	0·549	5·33	0·061	6·15
Average for all cow milk samples.	202	0·545	5·27	0·072	6·34
		Buffalo milk samples			
Murrah	22	0·543	5·50	0·060	6·31
Nagpuri	4	0·542	5·39	0·065	6·27

TABLE III—*contd.*

Summary of results for freezing point, lactose and chlorine of milk samples from different farms in India—contd.

Breed	Number of samples examined	Freezing point —°C.	Lactose monohydrate per cent	Chlorine per cent	Lactose chlorine number
Surti	11	0·546	5·43	0·060	6·24
Average for all buffalo milk samples.	37	0·544	5·47	0·060	6·28

DISCUSSION

Altogether 202 cow and 37 buffalo milk samples have been analysed. As already indicated, these samples were all preserved with mercuric perchloride. The interval between their collection and analysis varied from 2 to 34 days in extreme cases. It was previously shown by Venkateswara Rao, Dastur and Dharmarajan [1950], that mercuric perchloride in the concentration of 0·075 per cent preserved the milk in a good condition for over a month without affecting the value of the constants studied here. The present data give valuable information about the average composition of genuine milk, especially when considered in conjunction with the results for fresh samples already described [Dharmarajan *et al.*, 1950]. The average depression in freezing point of cow and buffalo milk samples varied between 0·541° to 0·551°C., with an overall average of 0·545°C. For milk samples reported in Part I of the present series, the average freezing point depression was found to be 0·548°C. Amongst the samples examined in the study here only about 3 per cent showed freezing point depression of less than 0·530°C. As previously recommended, this value can therefore with advantage be adopted for establishing the purity of market samples.

A glance at the data for lactose content of milk of samples in Tables I and II reveals that the values were higher than those obtained for Bangalore milk samples. The average lactose per cent in the milk of different breeds of cows varied between 5·09 and 5·53, with an overall average of 5·27. In contrast, milk samples at Bangalore had given an average of 4·92 per cent. The average for buffalo milk was also slightly higher than the previous results, namely, 5·47 per cent, compared to 5·25 per cent.

Milk samples studied here have given noticeably low values for chlorine content. In the previous paper [Dharmarajan, *et. al.*, 1950] with herd milk samples collected at Bangalore the chlorine content obtained was 0·089 per cent for cow milk and 0·066 per cent for buffalo milk. The average obtained in the present study was 0·072 per cent for cow milk and 0·061 per cent for buffalo milk.

The lactose-chlorine number of samples gave an average value of 6·34 for cow milk and 6·28 for buffalo milk. These values were very close to those given by Davies [1939], but slightly higher than the previous results with Bangalore samples.

Summary

Two hundred and two samples of cow and thirty-seven samples of buffalo milk collected from different parts of the country were analysed for freezing point, lactose and chlorine. The average freezing point depression of all the samples was 0·548°C., no difference being noticed in the values for cow and buffalo milk samples. Nearly 97 per cent of the samples gave a freezing point depression higher than 0 530°C. The average lactose and chlorine contents were respectively 5·28 per cent and 0·072 per cent for cow milk, and 5·27 per cent and 0·061 per cent for buffalo milk.

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GEOGRAPHICAL DISTRIBUTION AND SEASONAL INCIDENCE OF MAIN CONTAGIOUS DISEASES AMONG BOVINES IN THE PUNJAB FROM 1944-45 TO 1949-50

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(With three text-figures)

THE information regarding the occurrence of main contagious diseases among cattle in the Punjab was published in *Indian Farming*, Volume 3, March, 1946. This, however, related to the Joint Punjab and for the period from 1932-33 to 1934-44. The data, now presented in this article, relate to the Punjab (India) and for a period of six years from 1944-45 to 1949-50.

The Punjab State now comprises of thirteen districts divided into two revenue divisions of Jullundur and Ambala.

The data have been collected from the reports of outbreaks received from the field staff through their respective Divisional Superintendents.

TABLE I

Incidence of the main contagious diseases in bovines year-wise

Name of disease	Number of outbreaks					
	1944-45	1945-46	1946-47	1947-48	1948-49	1949-50
Rinderpest	581	725	759	1,573	777	127
Haemorrhagic septicaemia	1,111	1,026	807	114	705	472
Foot and mouth	147	517	718	88	296	1,067
Black quarter	115	59	36	5	23	11

From Table I presenting data for 6 years and Fig. 1 showing yearly distribution of contagious diseases since 1932-33, it is observed that rinderpest occurs in cycles. The first peak occurred in 1932-33 and 1933-34 ; second peak occurred after three years, i.e. in 1937-38 and 1938-39 and the third peak began in 1944-45, which lasted upto 1947-48 and since then it is on the decline. From the trend the next peak cycle can be predicted with some accuracy and thus mass prophylactic vaccination against rinderpest could be carried out a year earlier to prevent its causing heavy losses.

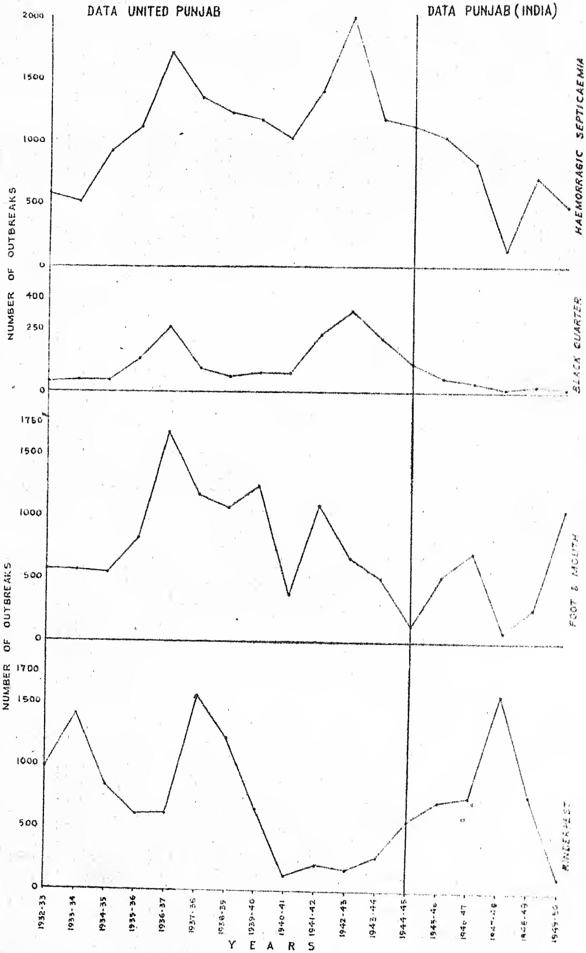

FIG. 1. Yearly distribution of contagious diseases.

The year 1947-48 was unprecedented in the history of the Punjab in which movement of livestock along with human population took place on mass scale due to the partition of the Province resulting in the spread of rinderpest also on mass scale throughout the State. The incidence of other contagious diseases, on the other hand, was lowest in the same year, most probably, due to the break down of communication and transport systems and preoccupation of the people in domestic worries due to the upheavel.

Great variation in the number of reports in the case of all other infectious diseases was observed from year to year, depending upon movement of livestock, rainfall and other environmental factors.

Lall [1946] reported that rinderpest was on the decline in the Punjab since 1932-33. However, observations recorded above do not agree with his findings. In so far as other diseases of bovine are concerned, the above observations are in conformity with those recorded by him.

Seasonal incidence

The number of outbreak reports of each disease received each month for all the years from 1944-45 to 1949-50 were pooled together to determine the seasonal incidence. Table II below shows the result.

TABLE II

Number of outbreaks of main contagious diseases in bovines month-wise

Name of disease	January	February	March	April	May	June	July	August	September	October	November	December
Rinderpest	914	277	206	269	161	257	236	244	288	338	654	695
Haemorrhagic septicaemia	246	156	120	91	79	73	235	1258	1178	100	183	207
Foot and mouth	43	26	78	101	826	819	218	90	121	102	63	47
Black quarter	8	11	10	30	17	25	20	19	33	22	12	12

Table II and Fig. 2 indicate that February and March are comparatively the healthiest months each year when the total number of reports of all epidemic diseases is the lowest.

Rinderpest. This disease is encountered more frequently during winter months, i.e., November to January.

Foot and mouth disease. The number of outbreaks of this disease begins to increase in early April, reaches its peak in May, stays in June and shows an abrupt decline from July onwards. In other words, with the onset of rains it declines fairly rapidly and is lowest during winter months.

Haemorrhagic septicaemia. The highest number of outbreaks of this disease occurs during and soon after the summer rainy season ; thus it is at its climax during the months of August and September and may commence as early as July and later

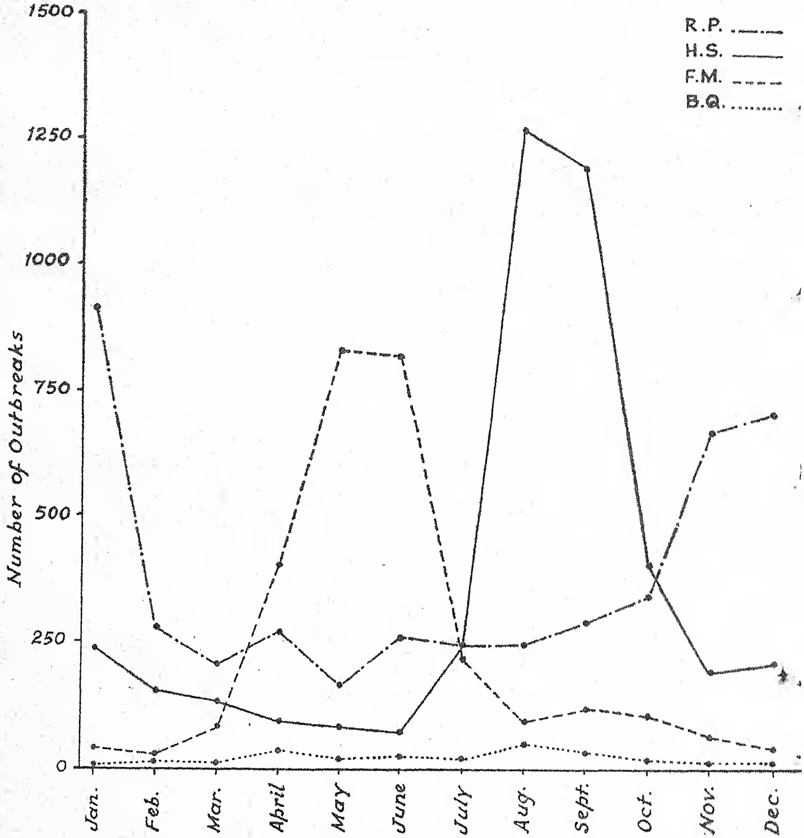

FIG. 2. Seasonal distribution of main contagious diseases in the Punjab.

extend to the month of October depending upon the length of rainy season. The disease also shows tendency to flare up occasionally after winter rains during the months of December and January.

Black quarter. In the case of black quarter, the outbreaks are fairly evenly distributed throughout the year but some increase is noticed during the months of August and September.

It is thus observed that each calendar year starts with rinderpest at its peak in January followed by comparatively healthy period for two months, i.e., February and March. Foot and mouth disease makes its appearance and holds sway from April to June, and then haemorrhagic septicaemia flares up with the onset of rainy season and holds the field upto September and October, when Black quarter also breaks out and takes its toll. In the end rinderpest starts increasing again in November and thus the cycle repeats itself each year.

The above conclusions in respect of various diseases are in agreement with those recorded by Lall [1946] except in the case of Foot and mouth disease, in which he mentioned that the disease started declining from June onward and was at low level in rainy season, where as observations recorded above indicate that the disease stays in June and July marks the decline of the infection and is low in winter months. In this respect observations recorded in this paper are in conformity with those reported by Hutyra and Marek [1949].

The above observations are supported by statistical analysis and finding the value of 't' and testing its significance.

In the case of rinderpest

Mean value for peak months of November, December and January . . . . =754·3

Mean value for other 9 months . . . . =253·2

$$t = \frac{754\cdot3 - 253\cdot2}{\sqrt{5887\cdot9 \times 0\cdot7}} \quad \ldots\ldots\ldots \quad = 9\cdot3^{**}$$

This is significant beyond 1 per cent level and thus the difference observed due to seasonal effect is real and is not a chance occurrence.

Haemorrhagic Septicaemia—

Mean value for peak months of August and September . . =1218

Mean value for other 10 months . . . . =179·9

$$t = \frac{1218 - 179\cdot9}{\sqrt{9246\cdot7 \times 0\cdot8}} \quad \ldots\ldots\ldots \quad = 13\cdot6^{**}$$

In this case also chance occurrence is much beyond 1 per cent level and the seasonal difference is highly significant.

Foot and Mouth disease—

Mean value for April, May and June . . . . . . =682

Mean value for other 9 months . . . . =87·4

$$t = \frac{682 - 87\cdot4}{\sqrt{14510\cdot3 \times 0\cdot7}} \quad \ldots\ldots\ldots \quad = 7\cdot0^{**}$$

Highly significant beyond 1 per cent level.

Table III indicates the distribution of contagious diseases district-wise. The number of reports of outbreaks received in case of each disease in each district is an average of last 6 years period. As the cattle population varies considerably from district to district, the number of outbreaks per 1,00,000 cattle was calculated to get comparative idea on uniform basis.

TABLE III

District-wise distribution of contagious diseases in bovines and effect of rainfall on them

Name of district	Total cattle population	Average rainfall per annum during last 6 years in inches	Average number of reports received per annum per 1,00,000 cattle				Total No. of out breaks per 1,00,000 cattle	Remarks
			R. P.	H. S.	F.M.	B.Q.		
Ferozepur	7,48,080	12·9	7·3	8·4	5·1	0·1	20·9	
Hissar	4,89,893	15·1	8·4	5·5	7·5	1·6	23·9	
Rohtak	5,33,948	15·9	6·8	7·8	7·8	2·1	24·5	
Gurgaon	5,28,146	21·2	7·5	6·6	8·1	1·3	23·5	
Karnal	7,43,823	21·8	10·6	3·7	5·8	0·4	20·5	
Amritsar	5,44,641	22·4	13·0	24·3	4·7	0·03	42·0	
Ludhiana	4,11,802	27·5	12·5	15·4	5·2	0·4	33·5	
Jullundur	5,03,529	29·0	12·6	14·2	11·0	0·5	39·2	
Hoshiarpur	6,26,416	36·1	11·4	12·7	4·2	0·4	28·7	
Ambala	5,16,808	40·2	10·0	8·9	11·3	0·3	30·4	
Gurdaspur	6,36,067	45·7	10·9	13·5	3·3	0·04	27·7	
Kangra	7,08,116	71·7	17·8	4·0	5·2	0·2	27·2	
Simla	21,417	39·0	24·8	0	88·8	0·8	114·4	

From Table III, it is observed that the first five districts have an average rainfall of 17·4 inches per annum and the total number of outbreaks is 22·5 per annum per 1,00,000 animals. In the next five districts the average rainfall is 31·0 inches per annum and the total number of reports is 34·8 per annum per 1,00,000 animals. In the last two districts the average rainfall is 58·7 per annum and the total number of reports is 27·5 per annum per 1,00,000 cattle. Thus moderate rainfall between 22 in. to 40 inches in the Punjab is more favourable for occurrence of epidemic diseases than rainfall below 22 in. and above 40 inches.

In so far as individual contagious diseases are concerned, rinderpest is observed to continue increasing with increasing rainfall; while on the other hand, Black quarter shows decline with increasing rainfall. The incidence of hemorrhagic septicaemia and Foot and mouth disease is more in medium rainfall as compared with both low and high rainfall. Table IV clearly illustrates the above statement.

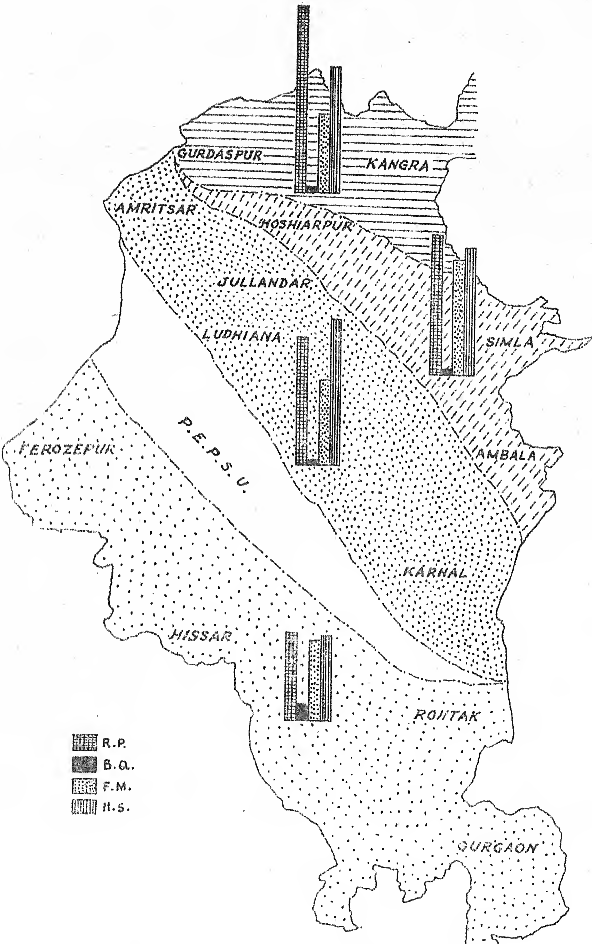

FIG. 3. Incidence of contagious diseases on regional basis according to the rainfall Histograme Scale 1 in. =400 outbreaks.

TABLE IV

Incidence of contagious diseases according to rainfall

Average rainfall in inches	Average number of reports per annum per 1,00,000 cattle				
	R.P.	H.S.	F.M.	B.Q.	Total
17·4	8·1	6·4	6·9	1·1	22·5
31·0	11·9	15·1	7·5	0·3	34·8
58·7	14·4	8·8	4·2	0·1	27·5

Effect of rainfall is also further apparent when the state is divided into four regions according to rainfall and the effect of rainfall determined on each disease separately.

1. Above 40 inches.
2. 30 in. to 40 inches.
3. 20 in. to 30 inches.
4. Below 20 inches.

The number of outbreaks in each region has been calculated per 1,00,000 cattle popultion in each region and is given in Table V.

TABLE V

Regional distribution of the State according to rainfall and its effect on incidence of contagious diseases

Regions according to rainfall	Number of reports per 1,00,000 cattle			
	R.P.	H.S.	F.M.	B.Q.
1. Above 40 in.	87	51	26	2
2. 30 in. to 40 in.	67	65	53	2
3. 20 in. to 30 in.	66	73	42	3
4. Below 20 in.	45	45	39	7

From Table V and Fig. 3 also it is observed that foot and mouth disease and haemorrhagic septicaemia outbreaks are more in region numbers 2 and 3 with rainfall between 20 to 40 in. than in regions 1 and 4 in which the rainfall is above 40 in. and below 20 in. respectively. Black quarter outbreaks are more in low rainfall region than in any other region. Rinderpest outbreaks are also lowest in region 4 with rainfall below 20 in., but it is higher in region 1 as well with rainfall above 40 in. which may, most probably, be due to greater susceptibility of hill cattle to this disease.

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STUDIES ON THE MANGE MITES OF LIVESTOCK IN INDIA

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SKIN diseases originate from various causes. In livestock in India the most important of them is the infestation with various types of mites. This is a serious problem for the veterinarian as a large number of livestock is infested with none or more species of mites and effective treatment entails considerable inconvenience. The disease is known under various names *viz.*, itch, scab, mange, scabies and a number of local names. About two dozen species or varieties of species are known to infest domestic animals and among them are some of the most serious pests of livestock. Pillers [1921] estimated a loss of 102,616 per annum due to mange in horses in the United Kingdom. To prevent this loss the United Kingdom and Canada have enforced legislation compelling livestock owners to report cases of mange in horses and sheep to the authorities for remedy.

Previous literature

Owing to the inaccessibility of literature an annotated review of the works of twenty-four authors on the subject is given.

Literature on mange reveals that life history of any of the burrowing mites causing scabies in man and animal has not been thoroughly worked out [Gordon *et al* 1943]; that work on treatment of various acariasis infection is very meagre and the stage in their life history when they are infective to healthy hosts is not yet known. The only available account is by Gordon *et al* [loc. cit.] who made limited observations on the life histories of *Notoedres* in white rats and *Sarcoptes scabei* in man and found that they are identical. They further suggested that the larval and nymphal stages are infective to healthy animals. Recently, Palimpsestov [1947] did valuable work on the subject and observed that sarcoptids of both sexes pass through four active and four passive stages. Mellanby *et al* [1942] in a series of controlled experiments proved that temperature had definite effect on the adult of *Sarcoptes scabei*. He recorded the thermal death point for these mites and showed that atmospheric humidity had no effect on it. But Troitzkii [1947] showed that air humidity had a great effect on the incidence of mange in sheep though air temperature had no effect on it. Gillain [1942] recorded that *Sarcoptes scabei* infection of pig could be experimentally transmitted to man and also recorded its spontaneous cure. Unsworth [1946] isolated demodectic mange parasite from ten per cent of the apparently healthy dogs. He failed to transmit demodectic mange infection by direct contact. Varenne [1946] reported successful transmission of sarcoptic mange from cattle to man. Imperial Chemical Industries (*Veterinary Bulletin*, 1950) record easy transmission of goat mange to man during cold months in Baluchistan.

Sen [1934] gave a review of the different methods of treatment of follicular mange in dogs. Basu [1944] gave a general account of the various types of mange mites in India along with their standard treatment. Roy and Ghosh [1944] found that oil of turpentine had properties lethal to *Saccoptes scabei.* Turpentine was found to reach and kill mites in their burrows without preliminary cleaning of the skin. Taylor [1945] showed that one per cent solution of Benzene hexachloride in olive oil or liquid paraffin was more effective than benzyl benzoate or tetraethyl thiurium mono-sulphide and DDT in the treatment of Notoedres acariasis. Psoroptes mange in cattle and horses were successfully treated with a sulphur lime dust by Gladenko [1946] and Fortushnuif [1947]. Tretyakova [1947] reported effective treatment of Psoroptes mange in horses with a powder made from a fused mixture of sulphur and naphthalene. Braun and Schuermann [1947] reported poor effect of Gesarol (DDT) on *Acarus scabei* and on scabies. The curative effect was, however, not superior to that of benzyl benzoate emulsion. Benzene hexachloride and Gammazene were found efficient in curing sheep scab [Kemper, 1948 and Dowhing, 1948], scaly legs [Griffiths and O'Rourke, 1949], sarcoptic mange in camels [Draz, 1947] and cattle mange (Brander). Phenothiazine ointment was found useful in treatment of demodectic mange [McCay and Udall, 1949]. Penicillin was successfully employed in treating demodectic mange [Floric and Gratecos, 1947].

Distribution and seasonal incidence

From the record of clinical material received from the various States and the reports of State Governments and of the Disease Investigation Officers it is clear that mange infestation is more or less common in livestock throughout India.

Regarding seasonal incidence of various species of parasitic acariasis causing skin diseases of livestock some preliminary observations were made at Izatnagar in 1947 and 1948 and it was found that mange infestation was definitely seasonal and restricted to a few months in the year *viz.*, October to March. *Sarcoptes scabei* var. *caprae*, the mite responsible for sarcoptic acariasis in goats was widely prevalent in our area (Izatnagar) and its incidence was very high during the winter months. Generally all types of mange infestation in livestock are worst in cold weather. The head and the ears are usually the initial points of infection. Later on, the disease extends backwards over the body and limbs. During the period October 1947 to February 1948 the incidence of mange observed in goats varied from 33 per cent to 77 per cent, the peak being in December. During this period out of 79 goats examined for mange 59 animals showed mange infestation. As regards the follicular mange infestation in dogs, 3 out of 16 dogs examined showed follicular mange infestation in 1948 in the month of November within the Indian Veterinary Research Institute premises. In all the three cases, the area affected was found hairless and the skin appeared deadened and grayish.

Some critical observations on various species of mange mites were made during the period November 1949 to March 1950 and their incidence in various species of animals is reported in Table I.

TABLE I

Incidence of mange at Izatnagar during November 1949 to March 1950

Species	November 1949		December 1949		January 1950		February 1950		March 1950	
	No. examined	No. +	No. examined	No. +	No. examined	No. +	No. examined	No. +	No. examined	No. +
Goat	10	1	39	10	36	6	16	3	29	2
Sheep	—	—	12	*nil*	21	*nil*	10	*nil*	9	*nil*
Cow	25	*nil*	45	,,	32	,,	38	,,	25	,,
Buffalo	12	1	27	1	47	,,	26	,,	26	,,
Horse	—	—	10	*nil*	10	,,	6	,,	6	,,
Dog	4	*nil*	17	1	21	2	6	1	5	,,
Cat	—	—	3	*nil*	4	*nil*	—	—	—	—

During the period, out of 130 goats, 52 sheep, 165 cows, 138 buffaloes, 32 horses, 53 dogs and 7 cats examined for mange, 22 goats, 2 buffaloes and 4 dogs were found positive. The infection in dogs was due to *Demodex folliculorum* and in the others *Sarcoptes scabei.*

Treatment

The standard treatments generally used against acariasis infection have been briefly stated by Basu [1944]. Recently, many chemicals of promise appeared in the market and some of them were tried to test their acaricidal properties. DDT, Derris and Gammexane (D.025—I. C. I.) were tried in this connection. A few years ago, there was a reference to the beneficial effect of sea water in the treatment of acariasis. Work was conducted to confirm it. Besides, a few other trials were made with varying percentages of the components of some standard dips.

The chemicals used were in the following concentrations :

1. DDT—in strengths varying from 0·1 per cent to 2·0 per cent.
2. Derris wash

Derries . . .	1 pound.
Soft soap . . .	4 ounces.
Water . . .	1 gallon.

3. Gammexane (D.025—I. C. I.)—Two per cent solution in kerosine oil.
4. Sea water and sodium chloride.
5. Tobacco lime infusion

Tobacco leaves . .	4 pounds.
Lime . . .	1 pound.
Water	50 gallons.

The treatment was tried mostly on *Sarcoptes* mange in goats, *Psoroptes* mange in sheep and *Demodex* mange in dogs.

Experiment 1

DDT.—(*a*) Three goats suffering from mange the causative organism of which was identified as *Sarcoptes scabei* var. *caprae*, were treated with a solution of two per cent. DDT in kerosine oil. The solution was rubbed thoroughly on the affected parts with cotton wool. A second application was made after seven days. The scrapings were examined every week and it was found that mites were present throughout the period of application. DDT was found unsuitable for treatment of sarcoptic mange in goats.

(*b*) An aqueous Suspension of DDT in 0·1 per cent and 0·5 per cent strengths was tried on sheep suffering from psoroptic mange. The former strength had practically no effect but the latter was found effective. In sheep dressed with 0·2 per cent DDT and above a slight irritation of the epidermis was noticed. No other constitutional disturbance was seen in any of them dressed with aqueous suspension of DDT.

Experiment 2

Derris.—(*a*) Three goats suffering from sarcoptic mange were treated with a wash containing Derris (with five per cent rotenone content) one pound, soft soap four ounces and water one gallon. It was rubbed thoroughly on the affected parts of the body with cotton wool. The scrapings were examined every week and it was found that the animals were free from mites for one week. When they reappeared in the second week, a second application of the insecticide was made which kept them again free for another week. Derris failed to cure goats suffering from sarcoptic mange.

(*b*) The above wash at a strength of 1·0 per cent was tried on two sheep suffering from psoroptic mange and cured one. Incidentally it may be mentioned that the same wash in 0·1 per cent and 0·2 per cent strengths proved effective against biting lice and sheep ked respectively. No harmful effect was noticed in any of the animals dressed with this derris soap wash.

Experiment 3

Gammexane.—Four goats were treated with a solution of 2 per cent Gammexane in kerosine oil. The affected parts were thoroughly rubbed with cotton wool. Scrapings were examined every week for a period of seven weeks. No mites were detected. Gammexane was found to be an effective drug against sarcoptic mange in goats. No constitutional disturbance in the treated animals was noticed.

Experiment 4

Sea water and sodium chloride.—Experiments on eight goats suffering from mange caused by *Sarcoptes scabei* var. *caprae* were conducted to test the efficacy of sea water or its constituent salts in the treatment of mange. Workers from Madras [Venkatachalam *et al.*, 1943] reported definite beneficial effects on the parenterial administration of sea water in sarcoptic and follicular mange in dogs. In our series of experiments, the goats were selected from a flock infected with mange and the scrapings from their skin were examined to determine the causative organism.

During the experiment the experimental goats were kept in separate sheds away from the rest of the animals to prevent extraneous contamination and reinfestation. Four of these received eight injections on alternate days of approximately 40 c.c. per hundred pounds body weight of sterile sea water which was obtained from the sea at Madras. The goats were weighed before and after the experiment. The quantity of sea water administered varied according to the body weight of the animal. Likewise, another group of four mange goats in two batches received a series of seven and six injections of a 2·3 per cent solution of sodium chloride in distilled water, *i.e.*, the strength in which sodium chloride was present in the sea water used. In both the groups, half the number of animals received the inoculum intravenously and the other half intraperitoneally. No improvement was noticed in the condition of the animals treated. All of them died later on due to continued ill health and resulting weakness. The result of this experiment is tabulated in Table II.

TABLE II

Effect of administration of sea water and sodium chloride on sarcoptic mange.

Group	Goat number	Body weight commencement of experiment	Number of injections received	Method of injection	Quantity of inoculum	Body weight after experiment	Result
Sea water group	26	42 lb.	8	Intravenous	17·5 c.c.	48 lb.	No improvement. Scrapings positive for Sarcoptes.
	40	40 ,,	8	do.	16·5 c.c.	47 ,,	do.
	41	44 ,,	8	Intra peritoneal	18·0 c.c.	40 ,,	do.
	64	26 ,,	8	do.	10·5 c.c.	26 ,,	do.
Sodium chloride group	3	36 lb.	7	Intravenous	15·0 c.c.		No improvement. Scrapings positive for Sarcoptes.
	63	24 ,,	6	do.	10·0 c.c.		do.
	60	32 ,,	7	Intraperitoneal	13·0 c.c.		do.
	70	18 ,,	6	do.	8·0 c.c.		do.

One dog suffering from follicular mange was given intraperitoneal injection of 15 c.c. of sterile sea water on every alternate day for 12 days with no improvement.

Experiment 5

Tobacco-lime infusion.—A series of experiments both in the laboratory and on a small scale in the field were conducted to evolve a suitable sheep dip with particular stress on the use of indegenous products for the control of common ectoparasites infesting sheep in this country. Sheep used in these trials were infested with mange mites, *Sarcoptes* sp. Biting lice (*Bovicola ovis*), sucking lice (*Linognathus* sp.) and sheep ked (*Melophagus ovinus*) were also present. The tobacco-lime infusion dressing was prepared as under and the quantity of water was increased or decreased according to the concentration desired.

Tobacco leaves . .	4 pounds.
Lime	1 pound.
Water	50 gallons.

Tobacco leaves and lime were soaked in water for 48 hours and the infusion wrung through a piece of coarse muslin. Nicotine content of the tobacco leaves was estimated at 2·3 per cent.

The tobacco-lime infusion at 1·0 per cent strength did not show any effect on mange mites or sheep ked but proved effective against biting lice. No harmful effect was noticed in any of the sheep dressed with this infusion.

Summary

Studies on the seasonal incidence and treatment of mange in various domestic animals have been conducted at Izatnagar and the following observations are recorded :

Mange infestation is definitely seasonal and is almost restricted to a few months in the year *viz.*, October to March, as observed at Izatnagar.

During the period November 1949 to March 1950, out of a total number of 130 goats, 52 sheep, 165 cows, 138 buffaloes, 32 horses, 53 dogs and 7 cats examined for mange, 22 goats 2 buffaloes and 4 dogs were found positive.

Sarcoptes scabei and *Demodex folliculorum* were the common species of mites responsible for mange in domestic animals at Izatnagar.

Different strengths of DDT, Gammexane, Derris root powder and tobacco-lime infusion were tried on *Sarcoptic mange* in goats and psoroptic mange in sheep. DDT had no action against sarcoptic mange mites but a 0·5 per cent suspension was found effective against psoroptic mange. A derris wash was practically ineffective. against *Sarcoptis* mites but was found successful in *Psoroptes* (one out of two tried) The wash in 0·1 per cent and 0·2 per cent strengths proved effective against biting lice and sheep ked respectively. A 2 per cent solution of Gammexane (D.025) proved to be an effective cure for sarcoptic mange in goats. Tobacco-lime infusion at 1·0 per cent. strength had no effect on mange mites or sheep ked but was effective against biting lice.

Intravenous and intraperitoneal injections of sea water and sodium chloride (2·3 per cent) had no beneficial effect on sarcoptic (8 cases tried) or demodectic mange (one case tried).

Mange infestation is more or less common in livestock throughout India.

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THE PHARMACOLOGY OF GIGANTIN*

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(With Plates VI—X)

THE crystalline active principle, 'Gigantin' first isolated and so named by Pitchandi [Pitchandi, 1948], is obtained from the latex, commonly known as madar juice, of *Calotropis gigantea*. All parts of the plant, the leaves, bark, flowers and root are used in the indigenous system of medicine as antispasmodic, alterative and nervine tonic [Watt, 1893]. The milk is used as a counter irritant and also in criminal poisoning for suicide or for producing abortion. The constitution of the active principle was studied by Pitchandi who assigned to it the following formula C_{24}'H_{36} O_9 as the tentative molecular formula.

A preliminary report on the pharmacology of gigantin had been made by the present authors [Rathnasabhapathy, Lakshman Rao, Krishnaswamy, David, Ishwariah ; 1949 and 1951] who obtained some evidence that its action on the circulation was one of stimulation due to a direct action on the musculature of the heart and blood vessels, similar to digitalis. Arrhythmic hearts became regular and failing hearts were found to revive under the drug and this led the authors to undertake the present work in the hope that the drug might be a very valuable and cheap substitute to digitalis if not better.

MATERIALS AND METHODS

Gigantin used in these experiments was prepared by the Chemist in this laboratory according to the method described by Pitchandi [1948] and made available in sufficient quantities. As it is insoluble in water, alcoholic solutions were made use of ·1 : 1000 solution was made in absolute alcohol and further dilutions were made with normal saline as and when needed.

Acute and chronic toxicity following intravenous administration was studied in dogs weighing 2 to 4 kg.

Dogs, cats, kittens, rabbits, and sheep were employed as experimental animals in this investigation. Effects on circulation and respiration were studied on dogs anaesthetised with 2·2 c.c. per kg. of paraldehyde orally and cats under intramuscular chloralose anaesthesia, 110 mg. per kg. dissolved in hot alcohol. Blood pressure was recorded on a kymograph after carotid cannulation and the recording of respiration was accomplished with the use of a tracheal cannula connected to a Marey tambour as usual.

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*Published from the Research Unit on Indigenous Drugs, Department of Pharmacology, Madras Medical College, Madras.

Perfusion of mammalian hearts and experiments with isolated bits of plain muscles as uterus and sheep's carotid artery were done in an improved type of apparatus, invented by Vareed and Venkatachalam [1943] which are in use in this laboratory for over ten years giving very satisfactory results. The heart perfusion apparatus consists of a double walled flask of two litres capacity and a double surfaced condenser about 90 centi-meters long. The heart cannula is fixed to the lower end of the condenser. Water at constant temperature is carried through the double surfaced condenser and the double walled flask. A thermometer inserted into the heart cannula registers the temperature of the perfusion fluid. Injections of drugs are usually made into the bulb of the heart cannula which has got a capacity of about 10 c.c. Drugs injected into the heart cannula are thus diluted ten times in the bulb.

The apparatus for perfusing isolated uterus, intestines, etc., consists of a jacketed cylindrical bath of 100 c.c. capacity with three-way stop cock at the bottom and a jacketed two litres flask, connected together. Water at constant temperature is run through the outer jackets. Drugs when added into the bath are diluted 100 times.

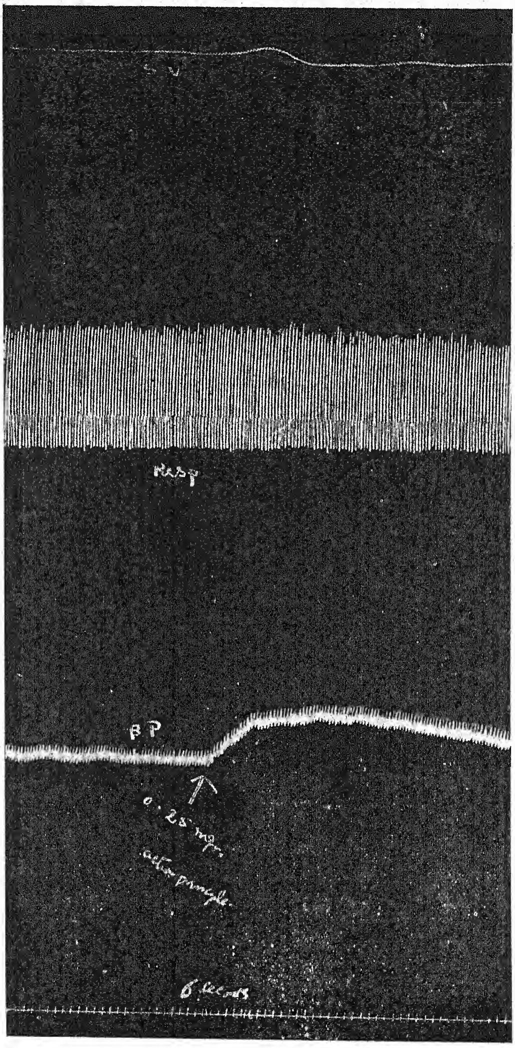
Perfusion of kidney was done in the Dixtion's apparatus.

Experimental data

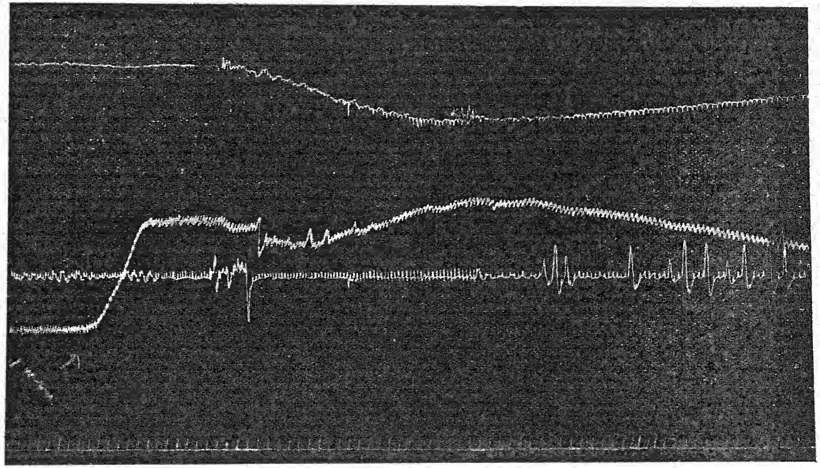
Toxicity. A precised L.D. 50 was not worked out. But it was found that a intravenous dose of 0·3 mg. per kg. was invariably fatal to majority of the dogs in 15 to 30 minutes. The toxic symptoms may be divided for convenience into three groups. In lethal doses there was frequent emesis of biletinged frothy fluid, distressed look, great exhaustion and death in about 15 to 20 minutes. The heart beat was highly accelerated with indistinct sounds resulting in fibrillation and cardiac failure. Pulse and respiration were accelerated throughout but respiration was dyspnoeic and oral.

On *post mortem* the liver was highly congested, enlarged and friable. The cortex of the kidney was slightly congested. There was nothing abnormal in other organs. All the tissues were subjected to histopathological examination. Except the liver and kidneys, all the other tissues were normal. In the liver there was widening sinusoidal spaces, the branches of the portal veins were engorged. But there was no definite change in the liver parenchyma. The blood vessels in the cortex of the kidney were engorged and the glomerular tufts were congested. Tubular epethelia showed clowdy degeneration.

In doses that are toxic, but not lethal, there was frequent emesis of bile-tinged frothy fluid, dyspnoea, distressed look, brady-cardia, weak pulse and accelerated respiration. But gradual recovery took place in an hour. In physiological doses, *i.e.*, 0·05 mg. per kg., there were no apparent changes in the rate of respiration and heart beat; but the beats were more powerful, pulse full and strong.



A record of the carotid blood-pressure, respiration and spleen volume of a dog of 2·4 kg., under paraldehyde anaesthesia. Note the persistent rise in the B. P. and the corresponding decrease in the spleen volume, after an injection of 0·25 mg. of gigantin. There is no change in respiration. S.V.—Spleen volume. Resp-respiration B.P.—Blood pressure. Time—6 seconds.



A record of the carotid blood-pressure, intestinal movements and kidney volume of a dog of 4 kg., under paraldehyde anaesthesia. Note the rise of blood pressure and the corresponding decrease in the volume of kidney after 1 mg. of gigantin intravenously. There is no change in the intestinal movements. Time—6 seconds. K.V.—Kidney volumes. I. M.—Intestinal movements. B.P.—Blood pressure.

TABLE I.

Controls with similar quantities of alcohol alone did not show any appreciable effect.

Serial number	Intravenous dose mg. per kg.	Number of experiments	Toxicity
1	0·3	6	Lethal in 50 per cent of the cases
2	0·25	2	Lethal in 50 per cent of the cases
3	0·20	2	Extremely toxic not lethal
4	0·15	2	Toxic
5	0·10	2	Less toxic
6	0·05	2	Physiological, non-toxic
7	Control 0·3 c.c. alcohol per kg.	3	Nothing abnormal

Gigantin in 0·05 mg. per kg. doses given intravenously and repeated daily at 24 hours' interval for 7 days did not show any indication of cumulative toxicity. The experimental animals were killed on the 8th day by ether. *Post mortem* and histopathological examination of tissues did not reveal any abnormalities.

HEART AND CIRCULATION.

Action on Blood pressure. A study of the effect of the drug on the general blood pressure of 16 dogs and two cats showed that there was always a sustained rise of blood pressure by about 10 to 15 m.m. of mercury, for about 30 minutes, after physiological doses ranging from 0·01 to 0·1 mg. per kg. (Plate VI).

In toxic doses, *i.e.*, 0·1 mg. per kg. and above, the blood pressure rose by about 15 m.m. of mercury above normal ; but it soon became irregular and suddenly dropped to the base line and was arrested.

To study the site of action of the drug a cat under chloralose anaesthesia was decerebrated by Elliot's method [1937] and a dose of the drug was given through the femoral vein. A sustained rise in the carotid blood pressure was noticed as before decerebration (Plate VII). An anaesthetised bitch weighing 2·3 kg. was sympatholysed by the administration of 15 mg. of ergotoxine ethane sulphonate and confirmed by giving 0·02 mg. of adrenaline, which produced the Dale's reversal. 0·25 mg. of gigantin given at this stage produced the usual rise of carotid blood pressure as before sympatholysis.

Action on the heart in situ

In a myocardiogram of an anaesthetised dog weighing 3 kg. gigantin in dose of 0·07 mg. per kg. stimulated all the chambers of the heart. There was an initial increase in the rate and amplitude of both the auricles and the ventricles followed by a slowing. The heart chambers returned to normal in about 30 minutes. Severing of both the vagi and atropinisation did not alter the action of the drug in any manner.

On Isolated hearts

Perfusion of two isolated kittens' hearts with 10 c.c. of 1 : 4,00,000 concentration of gigantin in the Locke's perfusion fluid followed by Normal Locke's did not produce any change in the rate of the heart beat but the amplitude was very slightly increased in the systolic level.

TABLE II

Gigantin on the rate and amplitude of isolated kittens' hearts

Concentration of the drug in the perfusion fluid.	1 in 4,00,000		1 in 2,00,000	
Time	*Rate*	*Amp.*	*Rate*	*Amp.*
Before drug	84	24	50	19
After drug	84	21	..	..
After 1 minute	84	25	70	21
After 2 minutes	84	25	80	23
After 3 minutes	82	27	65	26
After 5 minutes	84	26	40	25
After 10 minutes	..	..	40	25
After 15 minutes	..	..	30	24

Rate :—Rate per minute. amp.—Amplitude in milli meters.

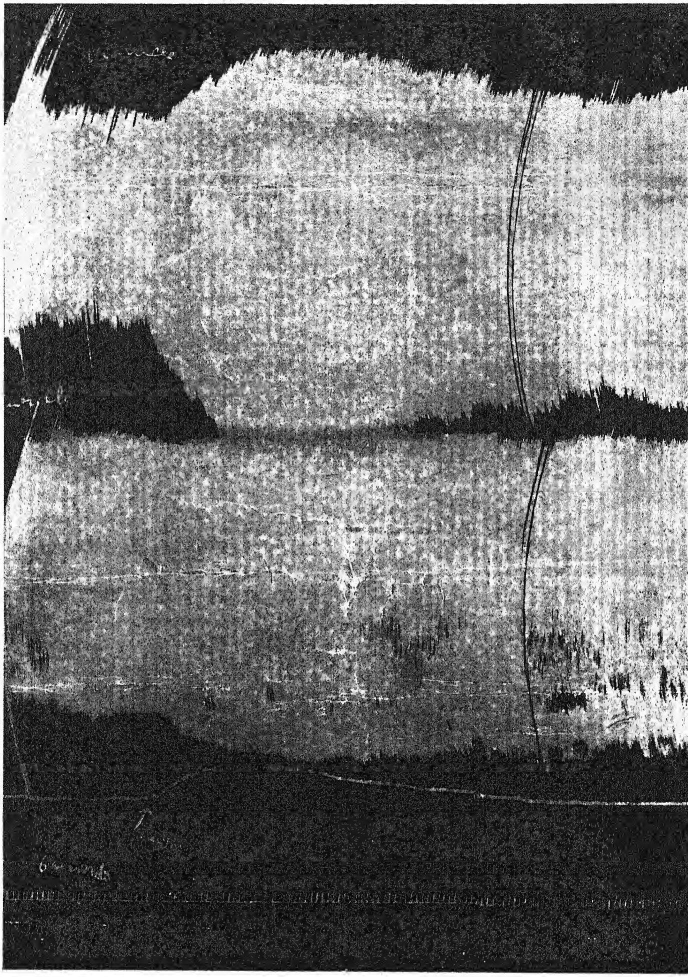
In physiological concentrations, *i.e.*, 1 in 2,00,000 in the perfusion fluid, there was an initial acceleration followed by a gradual slowing. The amplitude was normal in the beginning but later increased to more as the rate was slowed. The increase in the amplitude was due to the increased systolic excursions of the lever. The diastole was either normal or slightly deficient (Table II, Plate IX).

In toxic concentrations of the drug, *i.e.*, 1 : 2,000 in the perfusion fluid, the same changes were noticed during the first five minutes. But soon after this, heart block developed resulting in intermissions of the ventricles which were beating once for every two or more auricular beats. The intermissions became more and more frequent and the ventricles were finally arrested in diastole. Ventricular febrillations were not un-common before death (Plate IX).

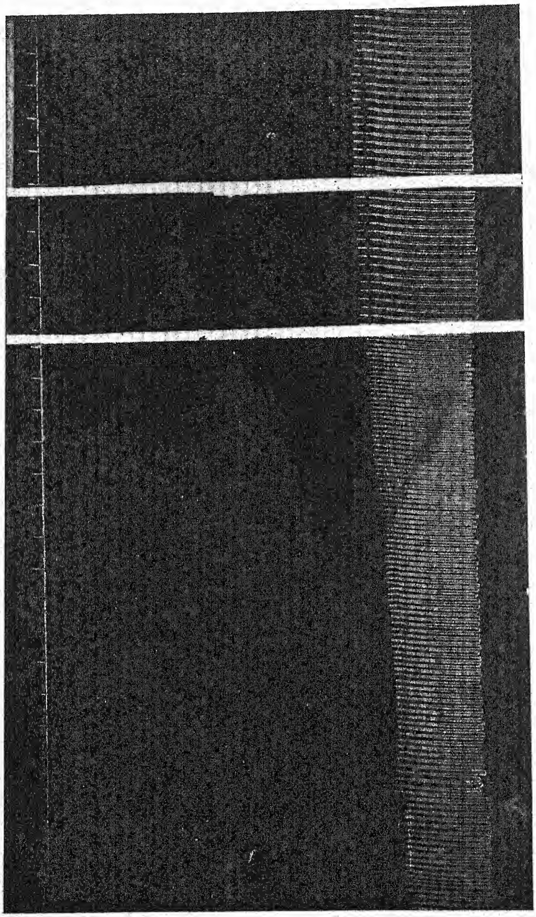
Auricular intermissions as those seen in the case of the ventricles were also noticed and they continued to beat for some time even after the ventricular stand-still.

Changes in rhythm and conductivity

Perfusion of isolated mammalian heart with 1 : 2,000 concentration of gigantin in the Locke's fluid caused a transient acceleration and then a slowing till the 5th



A record of the myocardiogram of a decerebrate cat under chloralose anaesthesia. Note the stimulation of the auricle and the ventricle and the rise in carotid blood-pressure after an injection of 1 mg. of gigantin. (Down strokes are contractions.) Time—6 seconds.



A record of the ventricular movements of a perfused kitten's heart, with 1 : 2,00,000 concentration of gigantin in the perfusion fluid.
(Time—6 seconds, up-strokes are systole.)

(a) Note the gradual increase in rate and amplitude.

(b) After 5 minutes. The rate is slowed, amplitude increased.

(c) After 10 minutes. Note the extreme slowing, long rest in the diastolic pause and steep and quick contractions.

minute. The auricles and the ventricles were beating in unision so far. After this the ventricles were reduced in rate, while the auricles were maintaining their normal rate. There was a single ventricular response for every two or more auricular beats. After some time, complete block developed resulting in the ventricles having their own rhythm and final arrest in diastole (Plate X).

The drug 1 : 2,00,000 concentration caused an initial increase, then a slowing of the rate of the heart. By perfusion with normal Locke's solution at this stage, apparently the normal rhythm of the heart was restored. But on repeating the dose, heart block was produced in three minutes, the ventricles responding for every two or more auricular beats only. The ventricles become irregular and were arıested.

Similar changes were noticed even in the auricles with regular or irregular intermissions. Finally, they were also arrested some time later than the ventricular stand-still.

It was found that the chambers responded to mechanical stimuli as a pin prick or pinching with fine forceps showing that the heart muscle was not dead and if only adequate stimuli reach them, from the pace maker they would continue to beat.

Contractility. In a physiological dose of gigantin, the heart was initially accelerated but the amplitude was diminished. This phase was only transient. Soon the rate was slowed while the amplitude was increased. The systolic contractions were quicker and steeper whereas the relaxations were slower than normal. The slowing was due mainly to a long rest in the normal pause. The increase in amplitude was due to systole, the diastole being normal. These changes in the contractility were observed in the auricles also but not to a marked degree as seen in the case of the ventricle (Plate IX, Fig. c).

Cardiac out-put. In a physiological dose of the drug, the rate of the heart was slowed but the amplitude and force of contraction were increased. The systolic contraction was marked with no change in the diastole. The long pause in diastole facilitated complete filling of the ventricle and the powerful systolic contractions emptied the heart perfectly. Thus the out-put per beat may be said to be increased. The slowing of the rate was thus compensated for by the increased pulse volume and the out-put per minute might not be altered considerably by the drug.

Fixation and Cumulative effect. A dose of 0·05 mg. of gigantin given in the bulb of the heart cannula slowed the heart and increased the force and contractions. After about 30 minutes the heart was apparently normal, when a dose of 0·025 mg. was injected again into the heart cannula. This repetition produced not only a slowing of the heart but also caused auriculo-ventricular heart block in about three minutes. The ventricles responded once for every 2 or more auricular systole. Finally due to complete block they developed ectopic beats and were arrested. Thus a return of the heart to apparent normality was not an indication of the complete elimination of the drug, but showed that some drug has been left behind and were eliminated very slowly.

Electro-cardiogram of normal dogs taken in all the three leads after 0·05 mg. per kg., of Gigantin intravenously showed only bradycardia.

On the vessels. Onchometric records were taken of the volumes of spleen and kidney. The spleen volume showed a slight fall (Plate VI). The kidney volume was also reduced to a slight degree. In larger doses a definite reduction in the volume of the kidney was observed (Plates VI and VIII).

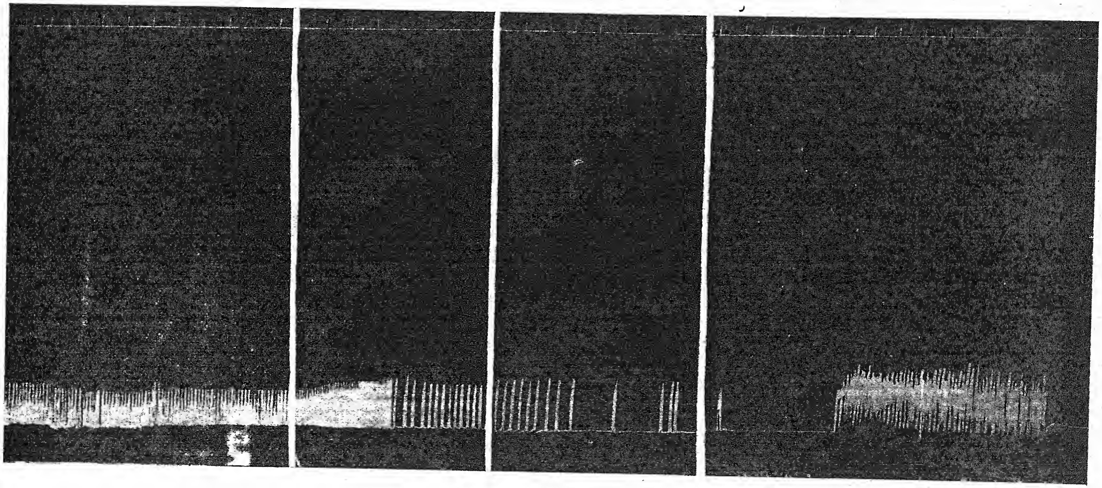
Liver and kidney were removed from the body and perfused using perfusion apparatus of Dixon at body temperature. Dogs under paraldehyde anaesthesia were bled through the carotid artery at the same time injecting saline through the femoral vein. When the fluid coming out of the carotid cannula became pale, the animals was opened up and the organ removed. This served to wash out the blood from the organs and prevented blood clotting inside. A cannula was placed in the main artery, any collateral branch was ligatured and the organ placed over a Buchner's funnel inside the Dixon's apparatus. The perfusion was then started with oxygenated Locke's fluid containing defibrinated blood from the same animal. The stroke of the pump was adjusted to a convenient height so as to deliver on an average about 0·5 c.c. per stroke. The perfusion fluid flowed out of the organ through the vein and was caught in the graduated vessel provided for the purpose. The time taken for the out flow of 50 c.c. was recorded by means of a stop-watch and when several readings were more or less constant a dose of gigantin was injected into the rubber tubing just above the arterial canuula. The time taken for the out flow of 50 c.c. was again recorded, several readings being taken in order to obtain the maximum effect. Table III gives the results of the experiment.

TABLE III

Organ perfused	Dose of gigantin in mg.	Average time for out flow of 50 c.c before injection	Maximum retardation after injection for 50 c.c.
		Minutes Seconds	Minutes Seconds
Liver	0·25	3-11	4-2
	0·25	7-13	2-37
Kidney	0·25	5-0	3-18
	0·15	5-52	0-7
	0·25	4-30	0-6

The out flow from the liver was markedly delayed with a dose of 0·25 mg. the maximum delay noticed being 4 minutes and 2 seconds. Same results were obtained with kidneys. Only in one case 0·25 mg. produced a maximum retardation by 3 minutes and 18 seconds. In all subsequent instances the retardation time was very little.

Perfusion of isolated kitten's heart through the coronary vessels was done to find out the effect on the coronary vessels. The out flow through the coronary veins was collected in a graduated apparatus for three minutes and measured. When



A record of the movements of the ventricle of a perfused kitten's heart, with 1 : 2,000 concentration of gigantin in the perfusion fluid. (Time—6 seconds. up-strokes are systole.)

(*a*) Immediate change.

(*b*) After two minutes. Initial increase, then a slowing.

(*c*) After five minutes. Note the partial heart block and ventricular intermissions.

(*d*) After 6 minutes. Note the complete heart block, ventricular fibrillation and arrest of the ventricle.

several readings were constant a dose of gigantin was given into the heart cannula and the amount of fluid collected for 3 minutes was measured for comparison. It was found that the drug did not produce any change in the volume of fluid collected in a unit of time as shown in Table IV.

TABLE IV

Organ perfused	Dose of drug given mg.	Venous out flow in 3 minutes before injection in c.c.	Venous out flow in 3 minutes after injection in c.c.
Kitten's heart	0·05	22	22
	0·05	22	22

Action of gigantion on isolated bits of arteries was studied using the carotid artery of sheep. This was obtained fresh from the slaughter house immediately after the sheep were slaughtered. The carotid artery was cut spirally to a length of about 1 inch and left in a constant temperature bath with oxygenated Locke's fluid for about 30 minutes. One end of the bit was tied to a fixed point below and the other connected to an Evan's frontal lever writing on the drum. The capacity of the bath was 100 c.c. 0·04 mg. of adrenaline added to the bath, constricted the arterial muscle by a nervous action. The bath was changed and 0·5 mg. of gigantin was added. The vessel constricted but the relaxation was very gradual unlike in the case of adrenaline. After changing the bath the nerve terminals of the vessel were paralised with ergotoxine-ethane-sulphonate by adding 0·18 mg. to the bath. The bath was changed and sympatholysis was confirmed by a dose of adrenaline added to the bath. But a dose of gigantin did produce a constriction of the vessels by a direct action on the arterial muscle.

Action on other plain Muscles

The intestinal movements of an anaesthetised dog was recorded by the balloon tambour method. A dose of 0·06 mg. per kg. given intravenously did not produce any appreciable change in the normal intestinal movements (Plate VIII).

Uterus. The record of the movements of an isolated uterus of rabbit in the constant temperature bath showed a good contraction both before and after sympatholysis with ergotoxine, when 0·5 mg. of gigantin was added to the bath.

Diuresis. Diuresis in an anaesthetised dog was measured both before and after gigantin. A volume 250 c.c. of a 10 per cent solution of cane sugar was given early in the morning by stomach tube and the animal allowed plenty of water to drink. It was anaesthetised by paraldehyde, the abdomen opened and the ureter of one kidney was cannulated and the urine made to drop on an Inchley's drop recorder, which was connected to a Mary tambour. Every drop was recorded on the drum and the time record was also made simultaneously. When the drops per minute were uniform a dose of gigantin was given intravenously and the number of drops per minute was counted after the drug.

Rate :—Rate per minute. amp.—Amplitude in milli meters.

It was found that there was a transient increase in the number of drops recorded after a dose of 0·25 mg. of the drug given intravenously. This however was not sustained.

Action on respiration

Respiratory records taken by tracheal cannulation showed that the drug did not produce any change in the normal respiration ; but where it was already depressed a stimulation was recorded.

Discussion

Gigantin given intravenously to anaesthetised dogs produced a persistent rise of blood pressure which is not abolished by ergotoxine or decerebration. From the above experimental data, it is thought that the rise is due to the action both on the heart and the splanchnic vessels.

The action on the heart is direct on the musculature and independant of the central or peripheral nervous mechanism as the same is produced in isolated and atropinised hearts. Both hearts *in situ* and isolated behave similarly to gigantin. In all doses the rate is accelerated in the beginning, later slowed. The amplitude is normal in the early part but later increased. The increase is due mainly to the increase in the systolic level, the diastole being either normal or slightly deficient.

The initial increase followed by as showing of the rate of the heart under gigantin may be due to some changes effected in the pace maker, for, the whole heart muscle is involved in this action. The irregularities of the rhythm in the toxic phase is probably due to the heart block, for the ventricles beat once for every two or more auricular beats. This is followed after a time by a complete block when the ventricles develop their own rhythm, become irregular and get arrested. The final standstill of the ventricles is due to the failure of effective stimuli reaching them and not due to the death of the muscle, since the muscle responds to mechanical stimuli even after the ventricular arrest.

The conduction thus is first impaired in the auriculoventricular bundle and at a later stage in the auricular fibres. The impulses started by the pace maker thus fail to arouse the auricle, as at a previous stage impulses which reach the auricles fail to reach the ventricles. The auricles and ventricles in the absence of impulses to contract develop ectopic rhythm and when a number of successive impulses fail, prolonged and final stand still results. Finally the pace maker may be slowed and arrested.

The two factors which comprise the action of gigantin are slowing and increased contractility. The slowing is mainly due to a long rest in the normal pause. The increased contractility is shown by the increased systolic level, the diastole being normal or very slightly deficient. Further the systolic contractions were also powerful and complete for in the individual beats after gigantin, the systolic excursion of the liver is quicker, steeper and higher than normal. When the diastole is normal, while the contraction is greater the extent of movement and the volume of blood expelled are of course greater.

In a physiological dose the work of the heart is greatly improved. Work done in a unit of time is the product of the rate of beat and pulse volume. When the rate is slowed under the influence of the drug, the out-put per beat (pulse volume) is the chief point at issue and this depends upon the relative development of systolic contractions and diastolic relaxations. If the contractions become more complete and relaxations unchanged the pulse volume is naturally greater. Though the out-put was not measured in these experiments, but only the muscular contractions it may be inferred that the pulse volume is increased correspondingly.

But the work done per minute might not be increased as the increase in pulse volume is compensated for by the slowing. In a diseased or irregular heart where more rest is ensured without affecting the minute volume, it may be said that the drug increases the efficiency of the heart. In toxic doses, there is no question of efficiency for when heart block develops it is obvious that the out-put per minute is definitely less.

Gigantin seems to posses cumulative properties for the same physiological dose repeated when the heart apparently returns to normal produces toxic symptoms. The apparent return of normality is therefore not an indication that the whole drug has been eleminated but something is left behind which in conjunction with a subsequent physiological dose, makes up a toxic dose. In addition the drug gets fixed in the heart muscle as seen by the persistence of the action of the drug on isolated perfused heart, even by subsequent perfusion with normal Locke's solution for a prolonged time.

As seen from the experimental data the rise of blood pressure may be ascribed partly due to constriction of the blood vessels of the splanchnic area. The volume of spleen and kidney showed a marked decrease. Perfusion of isolated organs, liver and kidney indicate a constriction of the vessels leading to a diminished outflow of the perfusion fluid from the veins. The action on the kidney vessels were however slight. The coronary vessels were neither dilated nor constricted. The action on the vessels is direct on the muscular tissue of the arteries and not nervous in action, as the same is produced in isolated bits of arterial muscles even after sympatholysis by ergotoxine.

To find out if it has got uniform action on all plain muscles, it was tried on the uterine and the intestinal muscles, and it is seen that it has direct action on the uterine musculature only and has no action on intestinal musculature.

The transient increase in diuresis produced by the drug is probably due to the concomittant rise of the general blood pressure and increased flow of blood through the kidneys which is not of great therapuetic value.

Gigantin has no action on normal respiration. But it has been observed that it stimulates the respiration both in rate and depth where it is already depressed.

The slowing of the heart, increased contractility, cumulation are all characteristic of the digitalis series of drugs and in these gigantin resembles them.

Summary

The Pharmacology of gigantin on the mammalian cardio-vascular mechanism has been studied in detail.

By a direct action on the musculature of the heart and the vessels it produce a persistant rise of blood pressure.

The efficiency of the heart is increased in physiological doses.

The action on the heart is analogous to digitalis.

Gigantin has also a direct motor action on the plain muscle or the uterus. But it has no action on the plain muscle of the intestines.

The Coronary vessels are not constricted by the drug.

It has no action on normal respiration.

Acknowledgments

The authors' thanks are due to the Indian Council of Agricultural Research under whose auspicies this work was carried out, and to the Director of Animal Husbandry, Madras, for his keen interest evinced in this work. They are deeply indebted to Sri K. Lakshman Rao, B. Sc. (Pharm.) Research Assistant (Chemical) for the valuable technical help in the preparation of gigantin and to Dr. Govinda Reddy, N.B.B.S., M.D. Professor of Pathology, Madras Medical College, Madras, for his help in the histopathological examination of various tissues in toxicity experiments.

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REVIEWS

EMBRYOLOGY OF THE PIG

By Bradley M. Patten

(*Published by the Blakiston Company, Philadelphia and Toranto P. P. XIII and 352 with 186 illustrations in the text containing 412 figures of which 6 are in colour. Price 35s.*)

THE pig has been a subject of extensive embryological research and there is already plenty of published material on the subject. But no other book deals with the subject so effectively as the publication under review. The present work has the merit of presenting in a simple form the fundamental processes of mammalian development based on pig with special emphasis on the sequence of embryological events. The fact that this is the third edition of the book is a clear indication of its popularity and increasing demand. In the present edition the subject matter has been brought up-to-date and a number of new illustrations including some excellent coloured plates have been incorporated to enhance the usefulness of the book.

The book is divided into 13 chapters of which 5 have been devoted to the early stages of development in mammals. The seventh chapter which deals with the structure of pig embryos of 9 to 12 m.m. in length containing several illustrations is an exceedingly interesting and instructive chapter. The remaining chapters describe the organogeny in a lucid form. An account of the histogenesis of bone has been included in the twelfth chapter. The last chapter contains a description of the development of teeth, face and jaws.

The bibliography given at the end of the text is quite comprehensive.

It is, however, felt that a chapter dealing with the delicate technique of preparing embryological material for laboratory study would have been a welcome addition.

To the students of Veterinary and allied sciences it should be an exceedingly useful publication. (C.V.G. C.).

ARTIFICIAL INSEMINATION AND ANIMAL PRODUCTION

By Dr. Sampath Kumaran

(*Published by F. E. Livengood, Mission Press, Jabalpore*)

DR Sampath Kumaran's book on *Artificial Insemination and Animal Production* is a recent addition to the very limited literature available on the subject which is authored and published in India. As such, his publication is welcome.

The author has taken considerable pains in giving a very exhaustive review of the work so far done on various phases of Animal Production including the use of the technique of Artificial Insemination, Physiology of reproduction, hormone therapy and breeding practices, etc. In covering such a wide field of subjects, he has not ignored to show the relative progress of work carried out in India. The book would be found informative to those who have interest in these allied subjects. To the undergraduate student, it would also be of considerable help as a reference book particularly in regard to the practical application of artificial insemination. I would, therefore, commend to every educational institution as also to students undergoing courses in Animal Husbandry to encourage such a publication by owing a copy of the book. (P.N.N.)

MGIPC—M—1 AR—30-12-52—830.

ORIGINAL ARTICLES

FIELD APPLICATION OF ARTIFICIAL INSEMINATION IN CATTLE—I*

By P. Bhattacharya and S. S. Prabhu, Animal Genetics Section, Indian Veterinary Research Institute, Izatnagar

(Received for publication on 15 February 1951)

ONE of the major obstacles in the way of rapid and all round improvement of cattle in this country is the paucity of superior bulls [*Royal Commission Report on Agriculture*, 1928 ; Wright, 1937]. The bulls issued for field use fall broadly under two main categories ; (i) those from recognized government or privately owned farms and (ii) those selected from certain breeding tracts. Although the selection of breeding sires on the basis of their phenotypic characters and/or their pedigree records, which in many cases are incomplete, is not a sound method, considering the performance of the average Indian cattle, it will be possible to effect a quick improvement in quality, if the total annual bull requirement can be met from the above two sources. As it happens, however, the availability falls far short of the actual requirement, resulting in the indiscriminate use of scrub bulls for 'settling' the cow. Consequentially, though the annual expenditure by the various State Governments and local Bodies on cattle improvement work is considerable, the resultant benefit accruing to the cattle industry as a whole is comparatively small. Indeed, so long as natural mating is continued to be practised, the present state of affairs in the country is bound to continue. The key to the solution of the problem affecting cattle improvement really lies in the wider use of artificial insemination.

In practical animal husbandry, artificial insemination was successfully employed for the first time by Ivanow [1907] in Russia ; and it has been extensively used since as an accepted method of breeding in many advanced countries. The special advantage, among others, of the technique is the opportunity it provides for the economic utilisation of the available semen from superior sires.

First organized attempts to study the applicability of artificial insemination in this country were initiated in 1942 at the Indian Veterinary Research Insitute, Izatnagar, under a scheme sponsored by the Indian Council of Agricultural Research. The scheme started functioning from November, 1942, and came to a close in the year 1945. The work has been subsequently continued in the Animal Genetics Section, Indian Veterinary Research Institute. During the tenure of the Council's scheme, various aspects of artificial insemination, such as training of bulls, collection of semen and its preservation, method of insemination, long distance transport, etc., were studied. The results have been reported to the scientific committees of the Council from time to time [Bhattacharya, 1942-1945 ; Guha, Kohli and Bhattacharya, 1947].

The results clearly demonstrated that there was not much difficulty in introducing this method in practical animal husbandry in India. But before its extensive

* The work has been carried out under a scheme of research financed by the Government of India from the ' Grow More Food ' fund.

use was taken up on a country-wide scale, it was, however, deemed essential to perform a larger number of inseminations under representative field conditions obtaining in the country. This was found necessary in view of the practical difficulties met with in the field [Vaidya and Bhattacharya, 1945]. With this end in view four regional centres under the control of the Animal Genetics Section, Indian Veterinary Research Institute, Izatnagar were established at the following stations:

(1) Calcutta Centre at Bengal Veterinary College (started functioning from November, 1945).

(2) Montgomery Centre at Sir Datar Singh's Farm (started functioning from December, 1945).

(3) Patna Centre at Bihar Veterinary College (started functioning from January, 1946).

(4) Bangalore Centre at Indian Dairy Research Institute (started functioning from January, 1947).

The Calcutta Centre and to some extent the Bangalore centre also worked under conditions obtaining in large cities; whereas the other two were, practically, under rural or semi-rural environments. The work at the first two centres materially suffered during the communal disturbances before and after the partition. The Montgomery centre had to be closed down in August 1947, owing to partition of India.

The results reported in this article cover the period starting from the opening of the centres upto the end of 1946.

Bulls used and their training

There were in all 26 bulls at the three centres (16 *Bos indicus* and 10 *Bos bubalis*). The *Bos indicus* bulls belonged to the following breeds: *Sahiwal*, *Hariana*, *Nagori*, *Tharparker* and *Indo-European Cross-bred*. The *Bos bubalis* bulls were of the *Nili* and *Murrah* breeds. The bulls were of different ages when brought into the scheme and with the exception of two, were previously used to hand service. No difficulty was experienced in ultimately training them to serve in the artificial vagina. It was observed, however, that older bulls took longer time for training. The three *Sahiwal* bulls purchased from Delhi were slow at service and as such were found to be unsuitable for artificial service.

Number of Collections

Full particulars of collections from the bulls stationed at the three centres are given in Table I. The details include information on the number of collections made, the average volume of semen obtained per collection from each bull, breed, and species. Out of the 27 bulls, 10 gave less than 10 collections, 3 less than 20, while the maximum number of collections constituting 87 per cent of the total number came from the remaining 14 bulls. The largest number of collections (84) were made from the *Tharparker* bull No. 57-2, stationed at the Patna Centre. Even this number falls short by twenty of the number of possible collections from a normal routine of two collections per week. Considering the number of bulls, the total of

TABLE I

Showing details of collections at the 3 Artificial Insemination Centres

Name of centre	Serial number of Bull	Brand number	Breed	Total number of collections per			Average volume (c.c.) of semen collection per		
				Bull	Breed	Species	Bull	Breed	Species
1	2	3	4	5	6	7	8	9	10
Montgomery	1	K 600	Sahiwal	17	95	95	2·71±0·11	3·72±0·11	3·72±0·11
	2	J77/2·8		30			3·04±0·23		
	3	M.12/1·2		28			4·16±0·19		
	4	M.24/2·8		20			3·68±0·18		
	5	M.P. 1	Nili	35	135	135	2·43±0·12	2·41±0·06	2·41±0·06
	6	M.P. 2		40			2·23±0·12		
	7	P. A.		60			2·49±0·11		
Patna	8	158—2	Tharparker	21	213	213	3·07±0·29	3·10±0·07	3·10±0·07
	9	57—2		84			2·90±1·05		
	10	7—2		73			3·23±1·25		
	11	12231		35			3·65±0·21		
	12	B. 441	Murrah	11	27	27	3·62±0·29	3·54±0·18	3·54±0·18
	13	B. 439		16			3·42±0·22		
Calcutta	14	R. Mult	Indo-European	20	76	107	2·41±0·16	2·41±0·16	2·56±0·08
	15	×-Bred 2	×-Breds	28			2·44±0·22	2·44±0·22	
	16	×-Bred 1	Do	28			2·35±0·13	2·35±0·13	
	17	Hiralal	Nagori	31	31	..	2·84±0·11	2·84±0·11	

'possible' number of collections works out at 1456. The number of collections actually made were 533, *i.e.*, less than one per bull per week. The low number was due mainly to the lack of adequate demand for semen, and also in some measure to the inherent defect in the bulls themselves, and their inconsistent behaviour after being in use for sometime. The actual number of bulls maintained at each centre could have been reduced considerably, if it had been possible to select more active and pretrained bulls.

Average Volume of Ejaculum per Collection

The average volume of semen per collection varied according to species and breed, and within a breed, from animal to animal. *Bos bubalis* bulls on an average gave $2{\cdot}63 \pm 0{\cdot}07$ c.c. of semen as compared to $3{\cdot}10 \pm 0{\cdot}05$ c.c. given by the *Bos indicus* bulls. The difference is statistically significant. In *Bos bubalis*, the average for the *Nili* breed was $2{\cdot}41 \pm 0{\cdot}06$ c.c. and for the *Murrah* breed $3{\cdot}54 \pm 0{\cdot}06$ c.c. per collection. In both the breeds within breed differences, however, are not significant. In the *Bos indicus* breeds, the *Sahiwal* topped the list with $3{\cdot}72 \pm 0{\cdot}11$ c.c.; next came the *Tharparker* with $3{\cdot}10 \pm 0{\cdot}07$ c.c. followed by the *Nagori* and *Indo-European Cross-bred* with $2{\cdot}84 \pm 0{\cdot}11$ c.c., $2{\cdot}43 \pm 0{\cdot}19$ and $2{\cdot}35 \pm 0{\cdot}13$ c.c. per collection respectively. Differences amongst breeds are statistically significant. The bull to bull differences amongst *Sahiwal* and *Tharparker* are also statistically significant. The highest average volume was given by a *Sahiwal* Bull (M 12/1·2) stationed at Montgomery centre ($4{\cdot}11 \pm 0{\cdot}19$ c.c.), the lowest by the *Indo-European Cross-bred* bull No. 1 from Calcutta with $2{\cdot}35 \pm 0{\cdot}13$ c.c.

With the *B. bubalis* bulls, Ayyar [1944] observed an average volume of 3·00 c.c. per collection in the 16 collections made from 2 bulls, while Shukla and Bhattacharya [1949] found the average volume from 24 collections to be $1{\cdot}84 \pm 0{\cdot}99$ c.c. The results reported herein are from 162 collections made from 5 bulls. The average worked out is slightly higher than that reported by Ayyar [1944] and significantly higher than the figure reported by Shukla and Bhattacharya [1949]. In case of *Bos indicus* bulls, the average volume for *Sahiwals* is given as $3{\cdot}80 \pm 0{\cdot}35$ c.c. per collection by Shukla and Bhattacharya [1949]. This figure is not significantly different from $3{\cdot}72 \pm 0{\cdot}11$ c.c. found in our study. The former figure is an average of 18 collections made from a single bull, while the latter is from 95 collections made from 4 different bulls. Data regarding *Tharparker* and *Nagori* and *Indo-European Cross-bred* bulls are new and have not been reported previously. No attempt is made here to compare the results with those of foreign breeds. In view of the small number of bulls studied under each breed, the conclusions drawn above should be taken as tentative subject to confirmation on the basis of further observations on a larger number of bulls.

Month to month variation in average volume in the different breeds is brought out in Table II.

Preservation of Semen

In the early stages of the work at the centres, the ejacula were preserved 'neat' and used as such. Diluted semen was used in two centres later on. At Patna, neat semen had been used throughout.

TABLE II

Showing month to month variation in average semen volume

Month	Centre						
	Montgomery		Patna		Calcutta		
	B. indicus (Sahiwal)	B. bubalis (Nili)	B. indicus (Thar-parker)	B. bubalis (Murrah)	B. indicus (Nagori)	×-bred I	×-bredII
December	3·3	2·9	1·7	..	1·8	..	1·8
January	2·3	1·9	1 6	..	1·6	..	2·1
February	2·5	2·1	2·4	..	1·9	..	1·2
March	2·6	2·8	2·3	..	2·0	..	2·0
April	3·4	3·5	3·5	..	2·8	2·4	2·6
May	4·5	3·4	3·2	..	3·0	2·6	2·1
June	4·3	1·6	3·0	..	2·4	2·8	1·8
July	5·1	1·9	3·3	2·5	2·9	3·7	2·1
August	4·4	2·6	3·7	3·9	1·7	3·4	3·3
September	3·4	1·7	3·6	4·0	1·9	2·8	2·4
October	5·5	2·3	4·6	3·7	3·0	2·9	2·6
November	3·6	2·5	3·7	3·5	3·5	3·3	2·8
December	3·0	2·9	3·2	3·7	2·1	3·0	3·2

Inseminations

The insemination was done by using an all glass syringe fitted with a long ebonite nozzle and a Russian model vaginal speculum. This method was used at all the centres in spite of the disadvantage and loss of time involved in disinfecting the speculum and nozzle after each operation. This is a serious consideration where very large number of animals are to be inseminated in a short time. However, this method was adopted as the number of animals coming to the centres during the year under report was never unwieldy and because of the special advantage offered by the method namely, the detection of disorders of the reproductive tract like vaginitis, cervicitis, etc., and finding out whether the semen deposited inside the cervix was later on expelled or not.

Complete details as to the number of inseminations performed from the different bulls at the three centres, including details regarding the number of inseminations per collection, are given in Table III. It will be seen from the table that on the whole a larger number of cows than buffaloes have been inseminated. It is of

interest to note that while at Montgomery, the cow to buffalo insemination ratio is in favour of buffaloes, at Patna it is contrarywise. At Calcutta, the number of buffaloes inseminated is negligible. These figures, however, should not be taken as depicting a true picture of the actual species-wise demand at the three centres. At Calcutta, for example, the absence of buffalo insemination is mainly due to the poor response of the purchased bulls. Two buffalo bulls were purchased from Rohtak and transferred to Calcutta. One of them was young and the other though mature and ready for service, possibly due to the sudden change in the environmental conditions, could with great difficulty be made to take notice of females even on heat. It was used to natural service and repeated attempts failed to train him to ejaculate in the artificial vagina. At Patna, only towards the end of the year as reported herein buffalo bulls could be procured for use. The situation at Montgomery may justifiably be taken as a true reflection of the popular demand. To start with, *Hissar*, *Sahiwal* and *Nili* semen was available without difficulty and in sufficient quantities to meet the local demand. Yet, only seventeen out of a total of 482 animals were inseminated with *Hissar* semen. The reason was each 'chak' had *Hariana* bulls of their own supplied by the Civil Veterinary Department, Punjab. The demand for *Nili* semen was great, as no bulls of this breed were readily available to the villagers and there were instances when the villagers brought their buffaloes on heat from over a distance of ten to twelve miles. The *Sahiwal* semen was in general demand as the farm, where the centre was located, had a reputation of possessing prepotent, pedigreed *Sahiwals*. The figures of this centre, therefore, may be taken as representing a cross-section of the actual demand in this tract. At Patna, only *Tharparker* semen was available. *Tharparkers*, though recommended by the provincial livestock authorities for general use, are not popular with the traditional *gwallas* in the city who show a strong fancy for cross-bred animals locally known as the 'Taylor breed'. If a bull of this breed was available to the centre, there is little doubt that the insemination figures would have considerably improved.

The largest number of *Bos indicus* females were inseminated from a bull stationed at Patna (*Bull No.* 57-Breed *Tharparker* with 384 inseminations). The largest number inseminated with semen from a buffalo bull was at Montgomery (*Bull No.* P. A. with 385 inseminations). It will be shown elsewhere that the figures could have been at least trebled, if sufficient number of animals were made available for insemination. Alternately, a fast moving vehicle at the disposal of the centre would have gone a long way in making larger number of inseminations possible.

From the economic aspects of the centres work, the figures given in Table III are revealing. It will be seen that at Montgomery, though ten bulls were at the disposal of the centre, the majority of the cows were inseminated from only four bulls. These four bulls accounted for 67·9 per cent of total number of animals served. At Patna, out of 1216 inseminations, four out of seven bulls accounted for 77·3 per cent of the total animals served. At Calcutta, three bulls out of the four served 84·9 per cent of the cows. These figures indicate that about two bulls of a given breed, provided they are found satisfactory for artificial insemination work, are adequate for a centre of this type.

As for the number of inseminations per ejaculum, as already explained, the limiting factor had been the small number of animals coming to the centre for insemination. Thus though 'neat' semen alone was used at Patna throughout, the

TABLE III

Showing details of inseminations performed at the 3 Artificial Insemination Centres

Name of Centre	Serial number of Bull	Brand number	Breed	Total number of insemination per Bull	Total number of insemination per Breed	Total number of insemination per Species	Average number of inseminations per collections per Bull	Average number of inseminations per collections per Breed	Average number of inseminations per collections per Species
1	2	3	4	5	6	7	8	9	10
Montgomery	1	K 600	Sahiwal	24(17)	465(95)	482(100)	1·41	4·89	4·82
	2	J 77/2·8		163(30)			5·43		
	3	M.12/1·2		164(28)			5·86		
	4	M.24/2·8		114(20)			5·70		
	5	H 161·17	Hissar	17(5)	17(5)		3·40	3·40	
	6	M. P. 1	Nili	69(35)	734(145)	734(145)	1·97	5·06	5·06
	7	M. P. 2		151(40)			3·78		
	8	M. P. 3		28(2)			14·00		
	9	M. P. 4		101(8)			12·63		
	10	P. A.		385(60)			6·42		
Patna	11	158—2	Tharparker	120(21)	961(213)	961(213)	5·71	4·51	4·51
	12	57—2		384(84)			4·57		
	13	7—2		359(73)			4·90		
	14	12231		98(35)			2·80		
	15	B. 441	Murrah	67(11)	189(36)	189(36)	6·09	5·25	5·25
	16	B. 439		88(16)			5·50		
	17	B. 1		34(9)			3·78		
Calcutta	18	R. Mult.	Indo-European ×-Bred	86(20)	374(76)	569(107)	4·30	4·92	5·32
	19	×-Bred No. 2		147(28)			5·25		
	20	×-Bred No. 1	Foreign	141(28)			5·04		
	21	Hiralal	Nagori	195(31)	195(31)		6·29	6·29	

NOTE.—Figures in brackets are number of collections.

average number of animals inseminated from an ejaculum was practically the same as at the other two centres using diluted semen. The average figure for three centres works out at about five animals per ejaculum. Even with the low rate of dilution followed by the centres namely 1: 3, this figure could easily have been trebled.

In Russia, the first country to explore and utilise extensively the immense possibilities of artificial service, the number of animals inseminated at each centre, after seven years of organised work, works out at 239 per centre [Badirjan, 1938; Neumann, 1939]. In Denmark, where the work was started in 1936 and the progress had been very rapid, the number of cows inseminated per bull in 1944 comes to 699 [Twinch, 1946]. In the United States artificial insemination bulls were serving 342,012 cows which works out at 526 cows per bull [Twinch, 1946]. In England, the technique became popular only during the later years of World War II. According to the figures published by the British Ministry of Agriculture [1946], between June, 1945, and June, 1946, on an average one bull was serving 142 cows. At the three Government of India Centres, with eleven effective bulls, 2198 inseminations in all were performed. Taking the service rate into consideration, which is 1·3 for all bulls, the number of cows per bull comes to 146. In view of the fact that it being only the first year's effort and that considerable time was spent in equipping the laboratories of the centres, training the bulls and in educating the conservative farmers to accept the new technique, the figure is very encouraging.

TABLE IV

Month to month variation in the total number of inseminations

Month	Cows			Buffaloes		
	Montgomery	Patna	Calcutta	Montgomery	Patna	Calcutta
December, 45	10	2	11	34	..	..
January, 46	8	29	13	39	..	..
February, 46	4	23	14	13	..	..
March, 46	33	23	26	31	..	..
April, 46	48	50	50	36	..	..
May, 46	73	89	75	23	..	..
June, 46	49	92	60	20	..	..
July, 46	58	115	56	34	1	..
August, 46	53	125	21	60	21	..
September, 46	40	113	26	117	44	..
October, 46	49	117	41	161	49	..
November, 46	48	83	64	159	35	..
December, 46	59	113	92	134	48	..

The figures in Table IV indicate the seasonal 'trend' in the activity of the centres. But as this is the first year's work, no attempt is being made to draw any conclusions at present.

Conception Rate

The success of the artificial insemination can be measured through the resulting conception rates. Rowson [1944] has described three ways of calculating conception rates :

(i) By adding all inseminations irrespective of whether the animals became pregnant or not, and dividing this number by the number of pregnant animals.

(ii) By adding up all the inseminations of the pregnant cows and dividing by the number of pregnant cows.

(iii) By calculating the percentage of conceptions resulting from the first, second and third, and subsequent inseminations.

American and British workers generally estimate inseminations required per conception on the basis of 'non-returns' i.e., on the number of animals not presented for 'repeat' insemination on completion of 5th month after insemination. In this country, however, it often happens that when a cow repeats heat after an insemination, she is taken to a private bull, even for a number of times, and is not brought for artificial insemination on account of recalcitrant prejudice. At other times, false information is given that she is pregnant from an insemination, so as to escape any further attempts for being convinced to take recourse to artificial insemination. It has therefore been thought best to use only the correctly checked actual calving data for all the inseminations made, for calculating the success of artificial insemination technique in field practice. Table V shows the number of services required per conception at the 3 artificial insemination centres. They were comparatively high for the Calcutta centre. The average overall rate for *Bos-bubalis* bulls was 1·39 as compared to 1·36 for the *Bos indicus* bulls. The difference is negligible. As the rate depends to a large extent on the individuality of the bulls and of the cows mated to them, no attempt has been made to test the differences statistically. However, among the *Bos indicus* bulls, the lowest rate was shown by *Sahiwal* bull No. K600 at Montgomery, and the highest was that by the *Indo-European cross-bred* bull No. 2 at Calcutta. Among the buffaloes, *Murrah* bull B439 at Patna had the lowest, and the *Nili* bull MP3 at Montgomery had the highest service rate.

Anderson [1939], after a careful examination of literature on reported fertility of bulls, concludes that when a single service or insemination was allowed during one heat period, highly fertile bulls when used with normal, regularly breeding cows, required about 1·5 to 2·0 services per conception and this should be regarded as normal. With the exception of the Calcutta centre, the number of services per conception at the other two centres are less than 1·5. As the animals inseminated usually came from the surrounding villages, this high fertility requires elucidation. The possible explanation might be found in the fact that the animals were brought

TABLE V

Showing details of number of services required per conception at the 3 Artificial Insemination Centres

Name of centre	Serial number of Bull	Brand number	Breed	Total number whose claving data are			Average service rate per conception per		
				Known	Unknown	Holding	Bull	Breed	Species
1	2	3	4	5	6	7	8	9	10
Montgomery	1	K 600	Sahiwal	24	0	21	1·14	1·34	1·33
	2	J. 77/2·8		142	21	102	1·39		
	3	M. 12/1·2		155	9	117	1·32		
	4	M. 24/2·8		103	8	79	1·34		
	5	H 161·17	Hissar	16	1	13	1·23	1·23	
	6	M. P. 1	Nili	69	0	51	1·35	1·42	1·42
	7	M. P. 2		• 183	15	100	1·36		
	8	M. P. 3		24	4	16	1·50		
	9	M. P. 4		78	23	53	1·32		
	10	P. A.		335	50	227	1·48		
Patna	11	158—2	Tharparker	120	0	92	1·30	1·25	1·28
	12	57—2		374	19	254	1·32		
	13	7—2		355	4	250	1·27		
	14	12231		97	1	81	1·20		
	15	B. 441	Murrah	67	0	50	1·34	1·32	1·32
	16	B. 439		88	0	69	1·25		
	17	B. 1		34	0	24	1·42		
Calcutta	18	R. Mult.	Indo-European × Breed	54	32	33	1·50	1·67	1·63
	19	×-Bred No. 2		113	34	62	1·82		
	20	×-Bred No. 1		109	32	67	1·63		
	21	Hiralal	Nagari	129	65	53	1·55	1·55	

to the centres from long distances and as such in a large number of cases, the inseminations took place during the later phase of oestrus. According to Hammond [1927] ovulation takes place 12 to 17 hours *post oestrum*. The sperm takes on an average $5\frac{1}{2}$ hours (lesser in heifers) to travel to the place of fertilization [Brester *et al.*, 1940]. They survive for about thirty hours in the female tract [Trimberger and Davies 1943 ; Laing, 1945], so that, if insemination is carried out towards the end of oestrum, there is a greater chance of fertilisation taking place than if insemination is performed during early oestrum. Laing [1945 *b*, *c*] observed consistently better service rate among the free service group, *i.e.*, where the bull was allowed to run with the herd, than in the hand service group, except during the winter months, when the duration of heat is short. He comes to the conclusion that the better conception rate in the free service group is due to the service synchronising with the end of oestrum, and thus close to ovulation. This conclusion is supported by much higher fertility in herds where the bulls always run with the herd, than in these where 'hand service' is practised, and the improved conception rates observed by the Russian workers using double spaced inseminations [Andreev, 1937 ; Kurillov, 1937]. In this connection, the possibility of a certain percentage of the cows brought to the centre receiving stray natural service either before, or after artificial service due to the carelessness of the owners, has also to be borne in mind.

Among foreign countries, the most successful results with artificial insemination are reported from Russia and Denmark. Smirnov-Ugrjumov [1945], reporting the work of an artificial insemination centre serving several collective farms in Russia, gives the percentage conceiving with fresh and stored (upto 24 hours) semen at 96·6 per cent. Sorensen [1938] reports a percentage of 87·6 with 1·68 inseminations per conception among 1,070 cows inseminated by him in Denmark. Holm [1945] gives a range of 58—96 per cent for 78 co-operative cattle breeding associations in Denmark, using artificial insemination and above 85 per cent in 60 out of these 78 associations. The high percentage of fertility observed in Russia might be in large measure due to the techniques employed (Double-spaced in inseminations and paper capsule methods of insemination). Twinch [1946] believes that the high fertility rates achieved in Denmark are due to the Sorensen insemination instrument and technique [Sorensen, 1946] *i.e.*, use of fresh semen, the sterility service which is allied with artificial insemination, and the prevalence of self-contained herds.

In the United States, Burch [1939] found among 439 dairy cows in the New York Dairy herds bred during the five months period from 15 November, 1938 to 15 April, 1939 with semen from seven bulls, an average service rate of 1·99. Henderson [1939] gives a service rate of 1·9 for 1,089 cows served by eight bulls. Bartlett and Perry [1939] discussing the five co-operative artificial insemination units including about 55,000 cows in New Jersey State, observed an average service rate of 2·0 for all inseminations performed. Davis and Williams [1940] found a variation of 1 to 4 inseminations per conception for different bulls with an average rate of 1·3. Perry and Bartlett [1945] mention a service rate of 1·7 for cows selected at random from a single artificial insemination association. Synder and Baltzer [1945] reporting the work of Michigan Artificial Breeders Co-operative Inc. with eighteen bulls supplying semen for 8,900 cows in eighteen local associations, found a fertility of 47 per cent at first insemination and a further 42 per cent at the second insemination. In England, the service rate for the Reading centre is given as 1·7 by Bartlett [1944]

for nine bulls, and for the Cambridge centre as 1·31 to 1·86 for five bulls by Towson [1944]. The average estimated fertility for all centres in England is given as 66·2 per cent by the figures published by the British Ministry of Agriculture [1946]. In Italy, Vighenzi [1946] states that in five years 5,796 inseminations were needed to fertilise 3,747 cows which give an average service rate of 1·6. The average service rates so far observed at the three Government of India centres are decidedly better than the American, British and Italian figures.

In Table VI is given a comparative study of the relative influence of volume, age and treatment of semen on fertility. The available data have been divided into two main groups according to species. Each in turn is further divided into two sub-groups, according to the volume of semen injected. Each sub-group in turn is arranged vertically into four main classes according to the age of the semen used. Figures for neat and diluted semen are presented separately.

From Table VI (on page 175), it will be seen that slightly higher fertility is obtained when 1·0 c.c. of semen is used instead of 0·5 c.c. This is true irrespective of treatment (neat or dilute) of semen. This is in keeping with the findings of Walton [1933] who observed a decrease in percentage of conception with a decrease to 0·5 c.c. or an increase to 5 c.c. above the optimum 1 c.c. and contrary to the observations reported by Kozlova [1935] and Herman [1939]. The former found no significant difference in the calving percentage of cows inseminated by the cervical method with 0·2, 0·5, 1·0, and 2·0 c.c. of semen, while the latter reported very little difference in the conception rates when quantities varying from 0·1 to 6·0 c.c. were used for artificial insemination.

Comparing on *like basis*, it is observed that, with a single exception (which may as well be due to the fewer number of inseminations included), the diluted semen gave better results than neat semen. Further, there was no apparent decrease in fertility with age of semen up to 76 hours at Montgomery and Patna centres. At Calcutta Centre, however, there is an indication of lowered fertility with the use of aged semen. The latter results confirm the findings of Milovanov and Kulesova [1945], who found a reduction of 15 to 20 per cent in the number of pregnancies after 48 to 96 hours of storage at 5° to 8°C, and Underbjerg, Davis and Spangler [1942] who, using both diluted and neat semen, found a marked increase in service rates with semen stored for more than 24 hours. As opposed to this, and in line with the findings at Montgomery and Patna Centres, Willett, Fuller and Salisbury [1940] observed no important deviation upto 96 hours, with semen stored in yolk-phosphate diluent. Their data covered 1,500 inseminations and as in our studies, only sperm not less than +++ motility rating were used. Salisbury [1941, 1943, and 1944] reported a high percentage of conception with semen stored in yolk-citrate and yolk-phosphate diluents for as long as 5 days; further, using different rates of dilution no detectable effect on fertility level with dilution rate as high as 1 : 100 (without the addition of sulpha drugs) was observed by Salisbury [1946]; Salisbury *et al.* [1943; 1945]. With the addition of sulpha drugs, the dilution rate could be further increased [Salisbury and Bratton; 1948]. In view of the fact that our data are incomplete in many respects, and the number of inseminations performed using stored semen is comparatively small, no conclusions are drawn at present on the influence of age of stored semen on fertility.

TABLE VI.

Showing relationship between volume, age and treatment of semen and fertility.

Species	Volume injected in c.c.	Name of A.I. Centre	Average Service rate per conception: 1 hour, Neat	1 hour, Diluted	Above 1, but less than 24 hours, Neat	Above 1, but less than 24 hours, Diluted	Above 24, but less than 48 hours, Neat	Above 24, but less than 48 hours, Diluted	Above 48, but less than 76 hours, Neat	Above 48, but less than 76 hours, Diluted
1	2	3	4	5	6	7	8	9	10	11
Bos bubalis	0·5	Montgomery	1·63	1·47	..	1·40	..	1·28	..	..
		Patna	1·29	..	1·36	..	1·29	..	1·38	..
		Calcutta	..	..	..	..	..	..	..	..
	1·0	Montgomery	1·40	..	2·20	1·57	..	1·23	..	1·32
		Patna	..	..	..	..	..	..	..	..
		Calcutta	..	..	..	..	..	..	..	..
Bos indicus	0·5	Montgomery	1·30	1·14	1·42	..	1·40	..	1·38	..
		Patna	1·30	1·26	1·38	..	1·29	..	1·32	..
		Calcutta	..	..	..	..	..	..	..	..
	1·0	Montgomery	1·18	..	..	..	..	..	..	1·23
		Patna	1·23	1·30	1·25	..	..	..	..	..
		Calcutta	..	1·66	..	1·46	..	2·59	..	2·16

SUMMARY

Results of 2,935 inseminations made from 601 ejaculates collected from thirteen *Bos indicus* and eight *Bos bubalis* bulls stationed at the Montgomery, Patna, and Calcutta field artificial insemination centres run by the Animal Genetics Section of the Indian Veterinary Research Institute are analysed and discussed.

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SEASONAL VARIATION IN 'REACTION-TIME' AND SEMEN QUALITY OF GOATS

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(With one text-figure)

In recent years several investigations have been carried out into the influence of seasons on the semen quality of farm animals. It has been found by Mukherjee and Bhattacharya [1947] and Shukla and Bhattacharya [1947] working with Indian bulls and rams respectively that the semen qualities of the animals vary in different seasons. They have found that high air temperature associated with high relative humidity and rainfall as found in autumn (*i.e.*, from August to October) has an adverse effect on semen quality of these two species of farm animals. Little information is available regarding the seasonal influence on semen quality of goats. Phillips *et al.*, [1943] studied the seasonal variation in bucks of Saanen and Toggenburg breeds at Maryland (U. S. A.). Their observations were, however, limited to only two bucks, one of each breed. In view of the scanty information available in respect of the indigenous goat, it was considered desirable to study the effect of different seasons on its semen quality.

Material and methods.

Experimental animals consisted of eight goats purchased from the local market. They were all mature bucks between two to three years of age at the commencement of the experiment. Before the commencement of the experiment they were trained to mount an anoestrous doe and ejaculate semen in artificial vagina. Throughout the experimental period the animals were kept under the same managerial and dietary conditions as reported for rams by Shukla and Bhattacharya [1947]. Two collections of semen at an interval of 15 minutes were made from each animal every fortnight for two consecutive years, and under the same climatological conditions under which Mukherjee and Bhattacharya [1947] and Shukla and Bhattacharya [1947] respectively observed seasonal variations in the semen quality of bulls and rams. Each semen sample was examined for the following characteristics :

(1) Colour and consistency.

(2) Volume of semen.

(3) Initial motility of spermatozoa.

(4) pH of semen.

(5) Sperm concentration per ml. of semen.

(6) Total number of spermatozoa and

(7) Percentage of abnormal spermatozoa.

4

Besides the above mentioned physical characteristics, 'reaction-time' of the animals was also noted as in rams [Shukla and Bhattacharya].

Colour and consistency were determined by the visual appearance of semen. Volume was measured upto 1/100 of ml. Initial motility was scored according to the criteria of Erb *et al.*, [1942]. Hydrogenion concentration of semen was determined by B. D. H. capillator. Sperm cencentration was determined by the usual haemacytometer method. For the determination of percentage of abnormal spermatozoa the method as adopted by Mukherjee and Bhattacharya [1947] was followed.

For the study of the effect of seasons on 'reaction-time' and semen quality, the local seasons were distinguished as follows :—

Autumn *i e* from August to October.
Winter ,, ,, November to January.
Spring ,, ,, February to April.
Summer ,, ,, May to July.

Results.

Monthly averages of 'reaction-time' and semen quality are presented in Table I. From the table it is apparent that there did not exist any relationship between the 'reaction-time' and the quality of semen produced by the goats. For instance, in 1945-46 the average 'reaction-time' in February, April and August was almost the same but the quality of semen in these months varied very greatly. Similarly, in 1946-47 the average 'reaction-time' in the months of February, March, August and September did not show wide variation, although there was conspicuous variation during these months in the semen quality.

The colour of the semen samples during the entire experimental period was creamy. The consistency of the samples, however, was either thick or thin. The table shows that sperm concentration and total number of spermatozoa for thin creamy samples varied from 1726 to 3240 millions per ml. of semen and 959 to 2092 millions per ejaculate respectively, while those in the thick creamy ones were 2329 to 4045 per ml. of semen and 967 to 2983 millions per ejaculate respectively. Thin creamy samples had on the average higher pH than thick creamy samples. There was, however, no appreciable difference in the initial motility of spermatozoa between thick and thin creamy samples.

Average monthly variations in 'reaction-time' and semen characteristics are presented in figure I. From the figure it is apparent that the curve for 'reaction-time' did not show any regular trend of variation from month to month. There is, however, an indication that the average 'reaction-time' of the animals in the second year of the experiment was lower than the first year. The curves for initial motility and pH did not show any marked monthly variation. The curve for volume showed a tendency to follow the curves for sperm concentration and total number of spermatozoa. Distinct monthly variations were seen in the curves of sperm concentration and total number of spermatozoa.

The curve for abnormal spermatozoa showed an opposite trend to that of sperm concentration, *i.e.* high sperm concentration was associated with decrease of abnormal spermatozoa and *vice versa.*

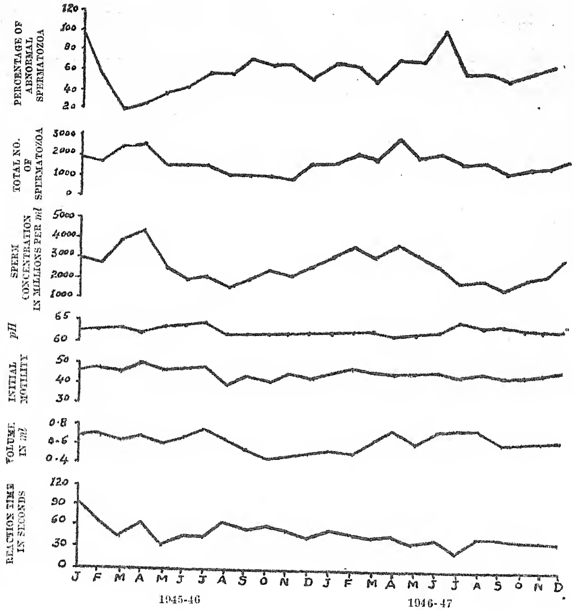
PERCENTAGE OF ABNORMAL SPERMATOZOA
TOTAL NO. OF SPERMATOZOA
SPERM CONCENTRATION IN MILLIONS PER ml
pH
INITIAL MOTILITY
VOLUME IN ml
REACTION TIME IN SECONDS
J F M A M J J A S O N D J F M A M J J A S O N D
1945-46
1946-47

TABLE I

Average monthly variation in 'reaction-time' and semen characteristics of goats

	1945-46											
	J	F	M	A	M	J	J	A	S	O	N	D
Reaction-time (in seconds)	98	68	44	66	30	43	45	66	58	62	59	45
Colour and consistency of semen	Th.C.	Th.C.	Th.C.	Th.C.	T.C.	T.C.	T.C.	T.C.	T.C.	T.C.	Th.C.	Th.C.
Volume of semen (in ml.)	0·68	0·72	0·64	0·69	0·61	0·67	0·76	0·65	0·57	0·46	0·49	0·51
Initial motility of spermatozoa	4·5	4·6	4·6	5·0	4·6	4·6	4·7	3·8	4·4	4·2	4·5	4·2
*p*H of semen	6·2	6·3	6·3	6·2	6·4	6·4	6·5	6·2	6·2	6·2	6·3	6·3
Sperm concentration (in millions per ml. of semen)	2967	2743	3804	4045	2595	2039	2023	1726	2149	2522	2314	2786
Total No. of spermatozoa (in millions)	1852	1799	2375	2524	1532	1412	1555	1078	1132	959	967	1532
Percentage of abnormal spermatozoa	11·1	5·4	2·4	3·0	3·9	4·4	5·8	5·9	7·2	6·7	6·7	5·2

	1946-47											
	J	F	M	A	M	J	J	A	S	O	N	D
Reaction-time (in seconds)	56	49	47	50	38	45	22	48	46	42	43	40
Colour and consistency of semen	Th.C.	Th.C.	Th.C.	Th.C.	T.C.	T.C.	T.C.	T.C.	T.C.	T.C.	Th.C.	Th.C.
Volume of semen (in ml.)	0·57	0·55	0·61	0·78	0·63	0·75	0·78	0·78	0·64	0·65	0·67	0·65
Initial motility of spermatozoa	4·4	4·8	4·6	4·5	4·6	4·7	4·5	4·7	4·5	4·5	4·5	4·8
*p*H of semen	6·3	6·3	6·3	6·2	6·3	6·3	6·5	6·4	6·5	6·4	6·4	6·4
Sperm concentration (in millions per ml. of semen)	3350	3718	3045	3812	3240	2784	1820	2064	1789	2014	2329	3226
Total No. of spermatozoa (in millions)	1770	2079	1641	2983	1823	2092	1718	1715	1153	1438	1447	1922
Percentage of abnormal spermatozoa	7·1	6·5	5·1	7·4	6·9	10·2	5·7	5·9	5·1	5·8	6·3	10·3

T.C.=Thin creamy.

Th.C.=Thick creamy.

Seasonal variation in average 'reaction-time' and semen quality of the goats are presented in table II. It will be seen from the table that the 'reaction-time' in summer was much less than in other seasons and there did not exist any relationship between the average 'reaction-time' and the quality of semen produced by the goats in different seasons. The average 'reaction-time' in summer was the least, while the quality of semen, judged by its colour, consistency and sperm concentration, was not better than in winter and spring. In autumn, winter and spring when there was no marked variation in the 'reaction-time', the quality of semen varied very greatly, autumn being inferior to that of winter and spring. The quality of semen in spring seemed to be better except in volume than in other seasons. Percentages of abnormal spermatozoa in winter, autumn and summer were higher than in spring. High percentage of abnormality in the ejaculates during winter was due to the presence of more bent tail spermatozoa and in autumn and summer due to tail-less ones.

TABLE II

Seasonal variation in avrage 'reaction-time' and semen charactristics of goats

	Autumn (August to October)	Winter (November to January)	Spring (February to April)	Summer (May to July)
Reaction-time (in seconds)	53·0	57·2	54·7	38·4
Colour and consistency	T.C.	Th.C.	Th.C.	T.C.
Volume of semen (in ml.)	0·63	0·60	0·67	0·71
Initial motility of spermatozoa	4·43	4·51	4·71	4·65
pH of semen	6·3	6·3	6·2	6·4
Sperm concentration (in millions per ml.)	2,044	2,829	3,528	2,417
Total number of spermatozoa (in millions)	1,246	1,582	2,235	1,689
Percentage of abnormal spermatozoa	6·26	7·84	4·79	6·12

T.C.=Thin creamy
Th. C.=Thick creamy

The summary of the results of analysis of variance of the data on 'reaction-time' and semen qualities is presented in table III. From the table it is apparent that goats varied in 'reaction-time' and all the characteristics of semen studied. Highly significant variations in 'reaction-time', pH of semen and total number of spermatozoa were found between the years. The variations in semen quality as observed among seasons (Table II) were real except in case of pH which was a chance variation. Variations due to interaction between seasons and years were highly significant for 'reaction-time' and pH of semen, and for initial motility it was only significant. Between months within seasons highly significant variations were found in sperm

concentration and total number of spermatozoa and significant variation in initial motility.

TABLE III

The results of analysis of data on 'reaction-time' and semen characteristics of goats

Factors for Analysis	Source of variation				
	Between Animals	Between years	Between seasons	Interaction between seasons and years	Between months within season
Reaction-time	**	**	*	**	0
Volume of semen	**	0	*	0	0
Initial motility	**	0	**	*	*
*p*H of semen	*	**	0	**	0
Sperm concentration	**	0	**	0	**
Total number of spermatozoa	**	**	**	0	**
Percentage of abnormal spermatozoa	**	0	*	*	0

** Significant at five per cent level
*Significant at one per cent level
0 Non significant

With a view to test the differences between goats, between years and between seasons, critical differences (C. D.) at five per cent level of probability of the variable characteristics were worked out. These are presented in Tables IV to VI. In the tables, the average figure under the same bar did not vary at five per cent level.

Table IV shows C. D. at five per cent level of the variable characteristics of semen between goats. It is apparent from the table that the average 'reaction-time' of goat 169 was significantly lower than of all others except 168. There was no significant variation between goats 168 and 164 but their 'reaction-time' was significantly lower than of goats 2, 160, 1, 137 and 3. Goat 3 showed the highest 'reaction-time'. The table also shows that there was no relation between the 'reaction-time' and the quality of semen produced by the goats. For instance goats, 2, 160, 1 and 137 did not vary in their 'reaction-time' but the quality of semen produced by them varied very greatly. Similarly, goats 168 and 169 did not vary in 'reaction-time', but their quality of semen as judged by volume, initial motility, sperm concentration and total number of spermatozoa varied significantly. Goat 3 as the table shows took maximum time to mount an anoestrous female but its semen quality was not poorer than that of others. The volume of semen of goat 164 was significantly lower than of the rest. Goats 160, 168, 137 and goat 1 had significantly lower semen volume than goat 2 and 169. The latter, however, had the highest semen volume.

Table IV

Critical difference at five per cent level of probability in 'reaction-time' and variable semen characteristics of the goat

Variable characteristics	Value of C.D.	Average of goats are arranged in ascending order.							
Reaction-time	16·3	169 (15·0)	168 (16·3)	164 (31·6)	2 (56·2)	160 (56·2)	1 (61·2)	137 (64·5)	3 (106·0)
Volume of semen	0·15	164 (0·29)	160 (0·53)	168 (0·53)	137 (0·56)	1 (0·61)	3 (0·63)	2 (0·78)	169 (1·12)
Initial motility	0·14	160 (3·50)	164 (4·50)	168 (4·65)	169 (4·70)	137 (4·73)	2 (4·83)	3 (4·85)	1 (4·86)
pH of semen	0·15	3 (6·2)	164 (6·2)	1 (6·3)	2 (6·3)	160 (6·4)	168 (6·4)	137 (6·5)	169 (6·5)
Sperm concentration	473	137 (2223)	169 (2272)	2 (2504)	160 (2841)	1 (2864)	168 (2885)	3 (3311)	164 (3755)
Total number of spermatozoa	500	164 (1076)	137 (1253)	160 (1505)	168 (1540)	1 (1712)	2 (1954)	3 (2085)	169 (2565)
Percentage of abnormal spermatozoa	2·46	169 (3·17)	2 (4·26)	1 (4·50)	137 (4·69)	168 (4·88)	3 (5·40)	164 (6·30)	160 (17·03)

Table V

Critical difference at five per cent level of probability between years

Variable characteristics	Value of C.D.	The averages of the years are arranged in ascending order	
Reaction-time	(8·2)	1946-47 (43·7)	1945-46 (58·1)
pH of semen	0·07	1946-47 (6·4)	1945-46 (6·3)
Total number of spermatozoa	251	1945-46 (1559)	1946-47 (1815)

TABLE VI

Critical difference of variable characteristics at five per cent level of probability between seasons

Variable characteristics	Value of C.D.	Summer	Autumn	Spring	Winter
Reaction-time	12·2	(38·4)	(53·0)	(54·7)	(57·2)
Volume of semen	0·09	Winter (0·60)	Autumn (0·63)	Spring (0·67)	Summer (0·70)
Initial motility	0·04	Autumn (4·43)	Winter (4·51)	Summer (4·65)	Spring (4·71)
Sperm concentration	473	Autumn (2044)	Summer (2417)	Winter (2820)	Spring (3528)
Total number of spermatozoa	355	Autumn (1246)	Winter (1582)	Summer (1689)	Spring (2235)
Percentage of abnormal spermatozoa	1·73	Spring (4·79)	Summer (6·12)	Autumn (6·26)	Winter (7·84)

As regards the initial motility of all the animals, goat 160 showed the least motility of spermatozoa. The sperm concentration of this goat was inferior to goat 3 and 164 which had the highest sperm concentration. The low initial motility of goat 160 in spite of high sperm concentration might be due amongst other causes to the highest percentage of abnormal spermatozoa found in this animal. Initial motility of goat 164 did not significantly differ from that of 168, but it was significantly lower than the rest, except goat 160. The low initial motility of this goat inspite of the highest sperm concentration than of the rest of the animals might also be due to the presence of a high percentage of abnormal spermatozoa which are likely to be less motile than the normal ones. Goats 2, 3 and 1 had high initial motility which might be due to the fact that goats 2 and 1 had high sperm concentration as well as low percentage of abnormal spermatozoa. The reason for goat 3 showing high initial motility inspite of high abnormality of spermatozoa might be its high sperm concentration. The higher initial motility of goats 137 and 169 in comparison with 160 and 164 might be due to their lower percentage of abnormal spermatozoa in spite of their lower sperm concentration than of the latter goats. It seems that initial motility rating, as has been done in this experiment by direct microscopical examination, depends not only on sperm concentration but also on the abnormality of spermatozoa. Abnormal spermatozoa are comparatively less motile than the normal ones and an increase or decrease in their numbers might also affect the motility of spermatozoa.

Average pH of semen of goats 3 and 164 was significantly lower than that of others. Associated with low pH, high sperm concentration was found in them. Again, goats 137 and 169 having higher pH than others showed the lowest sperm concentration. It seems, therefore, that high pH was associated with lower sperm concentration in goats.

Total number of spermatozoa has been calculated by multiplying sperm concentration per ml. of semen and the total volume of the ejaculate. It was expected, therefore, that the goat having the smallest semen volume but higher sperm concentration would show the lower total number of spermatozoa. This has been found to be true in case of goat 164. Goat 169 had the highest volume of semen and inspite of its low sperm concentration it had the total number of spermatozoa significantly higher than all except goat 3. Goats 160, 168 and 1 did not vary in their total number of spermatozoa as there was neither significant variation in their total volume of semen nor in their sperm concentration. Between goats 2 and 3 there was no significant variation in total number of spermatozoa probably because the difference in their volume was very little and non-significant ('although there was significant variation in their sperm concentration').

Critical difference at five per cent level of variable factors between years is presented in Table V. From the table it is observed that during the second year of the experiment the goats took significantly lesser time to mount an anoestrous female and ejaculate semen in artificial vagina. This observation is in complete agreement with that in rams by Shukla and Bhattacharya [1947]. The semen samples in the first year had lower pH than in the second year, but the total number of spermatozoa in the second year was significantly more than in the first year. Shukla and Bhattacharya [1947] found that in rams semen quality deteriorated in the second year of the experiment as indicated by higher pH, lower sperm concentration and total number of spermatozoa. There is, however, no such clear indication in this experiment.

Critical difference at five per cent level of 'reaction-time' and semen characteristics between seasons is presented in Table VI. From the table, it is evident that in summer, the 'reaction-time' was the least of all the seasons. There was, however, no relationship between the 'reaction-time' and the quality of semen produced by goats in differenet seasons. Volume of semen in winter was significantly lower than in the other seasons. Semen samples as judged by initial motility, sperm concentration and percentage of abnormal spermatozoa, were found to be the best in spring and worst in autumn.

Discussgin

Reaction-time. The average 'reaction-time' in goats has been found to be higher than that observed in rams by Shukla and Bhattacharya [1947]. This variation in 'reaction-time' might be due to the difference in species between goats and rams. As found in rams, there did not seem to exist any correlation between the sex-drive and sperm production. Summer which is the period of decline in sperm concentration shows lower average 'reaction-time' and in winter which is next best season for average sperm concentration records the highest 'reaction-time.'

Volume of semen. No work has been done on seasonal influence on the volume of semen in goats, excepting that of Phillips *et al.*, [1943] who used only two bucks in their experiment. The data, therefore, could not indicate clearly the seasonal trend. The results obtained in this study are not in agreement with the observations of Phillips and his co-workers. According to them highest average volume was obtained in fall but in this experiment highest average volume was found in summer. Spring was found to be the worst period by Phillips *et al.*, for semen volume whereas in this experiment the lowest semen volume was observed in winter.

Initial motility. Results obatined in the present experiment on seasonal influence on motility of spermatozoa is in general agreement with those found in rams by Shukla and Bhattacharya [1947]. But they differ from the findings of Phillips and his co-workers [1943] in goats who obtained highest average initial motility in fall and lowest in winter. In this experiment spring which showed highest initial motility was also found to be the best season for sperm concentration. Autumn was found to be the worst season for initial motility as well as for sperm concentration. Initial motility which has been estimated in this experiment by direct microscopical examination is the most empirical method of determining motility. It is dependent not only on sperm concentration but also on abnormal spermatozoa as found in some of the individual goats. Further more, cold air temperature as obtained in winter decreases the initial motility. As between winter and summer there was no significant variation in sperm concentration and percentage of abnormal spermatozoa, the low initial motility in winter might be due to cold environmental temperature.

Hydrogen—ion-concentration. The average *p*H of goat semen was slightly higher than that of ram as observed by Shukla and Bhattacharya [1947]. From the records of individual goats it has been found that like ram semen samples with high *p*H values were generally associated with poorer semen quality and *vice versa*. It was expected, therefore, that when there was some correlation between *p*H and sperm concentration in individual animals, a significant seasonal difference should have been observed in this factor as in case of sperm concentration. But in the present investigation *p*H did not show significant seasonal variation. There are two plausible explanations for this result. Firstly, it has been stated by McKenzie and Berliner [1937] that a rough co-relation exists between *p*H and sperm concentration and one cannot expect a significant coefficient of co-relation between the two. Secondly, in the present study *p*H was determined with B. D. H. capillator by colorimetric method which cannot give very accurate results.

Sperm concentration. Results obtained on the influence of season on sperm concentration are in general agreement with those obtained by Phillips *et al.*, [1943], except that according to them summer was the second best season, whereas in this case winter has been found to be the second best season. The results agree with those reported with rams by Shukla and Bhattacharya [1947] and with bulls by Mukherjee and Bhattacharya [1947]. These authors carried out their experiments under the same climatic conditions in which this experiment was conducted. It seems, therefore, that the slight difference in the results obtained in this experiment from those of Phillips and his associates might be due to the difference in the climate of the two places.

Total number of spermatozoa. The result of analysis of data on total number of spermatozoa showed that it was lowest in autumn and highest in spring. This result agrees with that obtained in rams by Shukla and Bhattacharya [1947] but is contrary to the findings of Phillips *et al.*, [1943] who obtained highest average total number of spermatozoa in summer and lowest in spring. The difference in our result from that of Phillips *et al.* might be due to difference in climate of the two places or due to limited number of data on which the results of Phillips and his collaborators were based.

Percentage of abnormal spermatozoa. Results obtained on seasonal variation of the occurrences of abnormal spermatozoa are similar to those reported in rams by Shukla and Bhattacharya [1947]. In summer and autumn the tailless spermatozoa and in winter the bent tail spermatozoa were more in the ejaculates. The bent tails have been indentified by Mukherjee and Bhattacharya [1949] as immature forms of spermatozoa. So it seems that in winter, when the rate of spermatogenesis was higher than in autumn and summer, more of these immature spermatozoa were carried into the male reproductive tract before they came to ampullae for ejectment. During autumn and summer, when the semen quality was found to be poorer, the tailless spermatozoa were found more in number and this might be due to high environmental temperature.

Summary

Seasonal variation in 'reaction-time' and semen characteristics of eight goats were studied for two consecutive years.

Highly significant variations were observed among goats in 'reaction-time' and in all the characteristics of semen studied except its *p*H which varied only significantly.

'Reaction-time' in summer was found to be significantly less than in the other seasons. 'Reaction-time' was found to be independent of sperm production.

Between years highly significant variations were found in 'reaction-time' *p*H of semen and total number of spermatozoa.

Between seasons highly significant variations were found in initial motility sperm concentration and total number of spermatozoa and significant variations in 'reaction-time', volume of semen and percentage of abnormal spermatozoa. The semen quality was found to be poorer in autumn and richer in spring.

Between months within season highly significant variations were found in sperm concentration and total number of spermatozoa and significant variation in initial motility.

Acknowledgment

The authors are indebted to Mr. A. Roy, Statistician, Indian Council of Agricultural Research for his guidance in the Statistical analysis of data presented in this paper. They are also grateful to Dr. D. P. Mukherjee of Animal Genetics Section, Indian Veterinary Research Institute, for many helpful criticism and assistance.

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SEASONAL VARIATION IN HAEMOGLOBIN AND CELL-VOLUME CONTENTS IN RAMS AND GOATS

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(With two text-figures)

Mukherjee and Bhattacharya [1947] have shown that there exists a seasonal variation in semen qualities and haemoglobin and cell volume contents of blood in bulls. Shukla and Bhattacharya [1947 and 1948] have also observed seasonal trend in the semen quality of rams and goats. The present study was undertaken to determine if the two blood constituents, *viz.*, haemoglobin and cell volume, in sheep and goat follow a seasonal trend as in bulls, and if so, how far this trend follows the seasonal changes in the semen of these species of animals as reported by Shukla and Bhattacharya.

Material and Methods

Blood samples were drawn from the jugular vein once a fortnight from each of the 9 rams and 8 male goats for determining the haemoglobin and cell volume contents. Haemoglobin was determined by the method of New Comer [1923] and cell volume by that of Napier and Das Gupta [1941]. During the entire experimental period the animals were kept under uniform nutritional and managerial conditions as have already been reported by Shukla and Bhattacharya [1947].

Results and Discussion

Month to month variations in haemoglobin and cell volume in the blood samples of rams and goats are shown in Table I.

Table I

Monthly variation in average haemoglobin and cell volume contents of blood in ram and goats

Months	Rams		Goats	
	Hb. gram per 100 c.c. of blood	C. V. Per cent	Hb. gram per 100 c.c. of blood	C. V. Per cent
January	7·77±0·27	34·83±1·38	6·79±0·20	31·67±1·29
February	7·35±0·23	34·62±0·71	6·63±0·66	31·74±1·30
March	7·91±0·23	34·12±0·93	7·09±0·13	31·01±0·57

TABLE I—*contd.*

Monthly variation in average haemoglobin and cell volume contents of blood in rams and goats

Months	Rams		Goats	
	Hb. gram per 100 c.c. of blood	C. V. per cent	Hb. gram per 100 c.c. of blood	C.V. per cent
April	7·95±0·18	33·88±0·71	7·05±0·12	30·95±0·82
May	8·62±0·21	34·93±1·69	7·36±0·14	31·37±1·10
June	7·51±0·27	33·70±1·09	6·36±0·11	29·46±1·10
July	7·02±0·18	33·24±1·11	6·32±0·16	28·49±0·91
August	6·52±0·15	31·83±0·86	5·94±0·14	27·71±0·79
September	6·78±0·23	32·15±1·02	6·19±0·27	27·86±0·83
October	7·47±0·17	34·89±0·91	6·62±0·27	28·65±0·79
November	7·69±0·13	35·22±0·31	6·40±0·17	29·57±0·86
December	7·66±0·16	35·16±0·88	6·60±0·21	30·50±0·53

It will be seen that in rams the variation in the mean percentage of haemoglobin was from 6·52 (±0·15) to 8·62 (±0·21) and that of cell volume from 31·83 (±0·86) to 35·22 (±0·31) and in goats from 5·94 (±0·14) to 7·36 (±0·14) and 27·71 (±0·79) to 31·74 (±1·30) respectively. The blood samples obtained from rams were richer in haemoglobin and cell volume contents than those from goats. In both the species, haemoglobin and cell volume were at their lowest level in the month of August. During this month, as seen from Table II, the average air temperature was high (85·9°F.) with almost highest relative humidity and high rainfall (6·11 in.).

TABLE II

Monthly variation in average air temperature, humidity and rainfall

Months	Average temperature (°F.)	Average percentage of humidity	Average rainfall (in inches)
January	58·5	71	1·32
February	64·2	63	0·75
March	75·3	45	0·36
April	86·7	22	0·00
May	92·7	31	0·01
June	93·5	50	5·25
July	85·6	81	16·89
August	85·9	80	6·11
September	85·4	65	4·01
October	79·0	72	5·17
November	68·9	64	0·01
December	61·7	62	0·21

For haemoglobin, the month of May was the best in both the species. Although the average air temperature was high (92·7°F.) during this month, the relative humidity was almost the lowest and rainfall negligible.

Data collected under Tables I and II are graphically represented in Fig. 1 and (Fig. 2). It will be seen from the figures that with the increase in the air temperature, humidity and rainfall there was a concomitant decrease in the haemoglobin and cell volume contents of blood in both rams and goats. The trend of variation in the two blood constituents due to external factors was almost similar to that of bulls as reported previously.

The data summarised in Table I were subjected to analysis of variance and the results of analysis are presented in Table III.

TABLE III

Summary of the analysis of variance of blood characteristics of rams and goats

Factors analysed for Variance	Source of Variation		
	Between Rams or between Goats	Between seasons	Between Months within season
Percentage of Hb.	** ††	** ††	** ††
Percentage of cell volume	** ††	** ††	° †

** Highly significant for rams
†† Highly significant for goats
° Not significant
† Significant for goats

From this table it is apparent that the variations observed in haemoglobin and cell volume among rams and goats were highly significant. In both the species, the variation in the two blood constituents due to season was highly significant. As in the previous study, the months have been grouped into four different seasons, *viz.* :—

August to October	Autumn
November to January	Winter
February to April	Spring
May to July	Summer

Highly significant variation between months within season was found in haemoglobin contents of blood in both rams and goats. Variation in cell volume between months within season was found to be significant only in the case of goats. In order to find out the most unfavourable and most favourable seasons for the

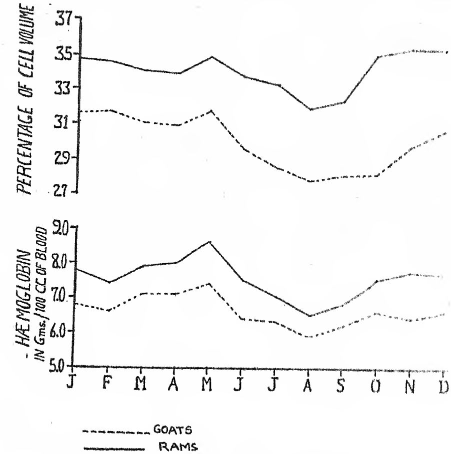

FIG. 1. Monthly variation in average haemoglobin and cell.

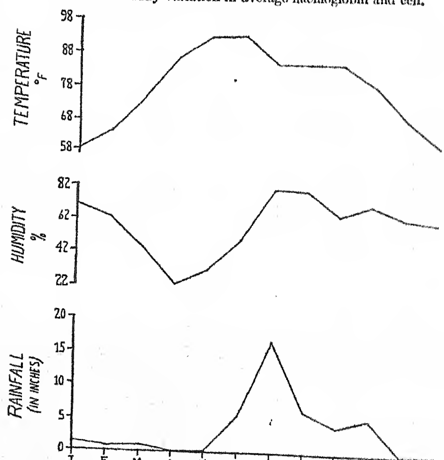

FIG. 2. Monthly variation average temperature humidity and rainfall.

two blood constituents, critical difference (C. D.) at five per cent level between seasons was worked out and the results are presented in Table IV.

TABLE IV

Critical difference at five per cent level of haemoglobin and cell volume contents of blood in rams and goats in different seasons

	Value of C. D.	Average of seasons arranged in ascending order			
Percentage of Hb. in blood of rams	0·24	Autumn (6·92)	Winter (7·71)	Summer (7·72)	Spring (7·74)
Percentage of Hb. in blood of goats	0·28	Autumn (6·25)	Winter (6·60)	Summer (6·69)	Spring (6·92)
Percentage of C. V. in blood of rams	1·22	Autumn (32·96)	Summer (33·96)	Spring (34·21)	Winter (35·07)
Percentage of C. V. in blood of goats	0·92	Autumn (27·87)	Summer (29·78)	Winter (30·58)	Spring (31·23)

In the table, seasons under the same bar did not vary significantly. It is seen from the table that the blood samples were poorest with regard to the two characteristics in autumn. In spring, the two blood constituents of goats and haemoglobin contents in rams were highest. Although, the percentage of cell volume in the blood of rams was highest in winter it did not differ significantly from that in spring.

Table V shows variation in average air temperature, humidity in different seasons.

TABLE V

Variation in average air temperature, humidity and rainfall in different seasons during the year 1946-47

	Autumn	Winter	Spring	Summer
Average air temperature (°F.)	88·43	63·03	75·40	90·60
Average humidity (in per cent)	69·00	65·66	45·33	54·00
Average rainfall (in inches)	5·09	0·52	0·57	7·38

Spring was characterised by a moderate air temperature and lowest humidity and rainfall, whereas, autumn had a very high air temperature with highest humidity and high rainfall (Table V).

Data regarding sperm concentration per c.c. of semen and total number of spermatozoa per ejaculate in different seasons, as obtained by Shukla and Bhattacharya [1948] are given in Table VI. It is evident from the table that like the blood constituents, the sperm concentration and total number of spermatozoa were also lowest in autumn and highest in spring in both the species.

TABLE VI

**Seasonal variation in sperm concentration and total number of spermatozoa in rams, goats during the year 1946-47*

	Rams		Goats	
	Sperm Concentration per c.c. of semen (in millions)	Total number of spermatozoa (in millions)	Sperm concentration per c.c. of semen	Total number of spermatozoa
Autumn	1919	1221	1950	1456
Winter	2752	1696	2968	1713
Spring	2814	1850	3525	2254
Summer	2362	1627	2615	1878

*Data obtained through the courtesy of Mr. D. D. Shukla and Dr. P. Bhattacharya

The results presented above show that the trend of variation in the two blood constituents and semen characteristics during different seasons in rams and goats were parallel. Furthermore, the trend of seasonal variation in the two blood constituents were the same as that reported in bulls by Mukherjee and Bhattacharya [1947]. Thus, it appears that the effect of season upon the blood and semen quality was similar in bulls, rams, and goats. In these species of farm animals, high air temperature associated with high humidity and rainfall as found in autumn brought about a deterioration in the semen and blood characteristics studied. In spring, when the atmospheric temperature was moderate with low humidity and scanty rainfall, the semen qualities improved and the blood also became richer in haemoglobin and cell volume. It seems, therefore, that the mechanisms by which air temperature, humidity, and rainfall affect reproductive function and blood constituents in bulls, also come into play in rams and goats.

Summary

Seasonal variation in haemoglobin and cell volume contents of blood from 9 rams and 8 male goats were studied for one year.

Haemoglobin and cell volume contents of blood were highly variable among animals and among seasons.

In both rams and goats haemoglobin content was found to be highly variable between months within seasons.

Cell volume of blood in goats was found to vary significantly between months within season but it was not so in case of rams.

It was found that in these animals, the seasonal trends in semen attributes and blood constituents were similar. The quality of semen and blood was found to be superior in spring, *i.e.*, from February to April and inferior in Autumn, *i.e.*, from August to October.

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AVIAN SALMONELLOSIS

STUDIES ON *SALMONELLA GALLINARUM*

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(With Plates XI to XVI)

The first outbreak of Salmonellosis due to a motile organism in a commercial flock of fowls in India was recorded in 1949 by Iyer *et al.* [1950]. Prior to this only once *S.gallinarum* was recovered from a single hen [Cooper and Naik, 1931]. However, duck Salmonellosis was reported in the Army Development Farms by Rao [1946]. Hitherto India had been free from *S. pullorum/gallinarum* infection in chickens though the disease is prevalent all over the world. An extensive survey initiated by this Section in 1945 revealed little evidence of its presence in most part of India. In the year 1950, three outbreaks of Salmonellosis occurred in widely separated areas in India. Two of these were due to paratyphoid organisms, while the third one was caused by *S. gallinarum*, the fowl typhoid organism.

Fowl typhoid is a disease of young adults rather than of old birds and much less of baby chicks. Brookes and Rhodes [1923] found that certain strains of *S. gallinarum* produced in young chicks lesions indistinguishable from those of *S. pullorum* infection. In North-Central U. S. A. the disease mainly affects young adults and rarely chicks [Bushnell, 1948]. In England it is most common in adults but has also been recorded in baby chicks [Menzies, 1947]. In South Africa the disease is erratic, attacking exclusively one breed on a farm or only chicks, and in others adults [Coles, 1948]. In Hawaii, sporadic outbreaks of acute form in chicks and chronic form in adults are reported [Fitzgerald, 1947]. In Australia adult mortality due to *S. gallinarum* is unknown [Leynen, 1939].

Beudette [1925], and Komarov [1932] isolated *S. gallinarum* from the yolks of chicks and ovaries of the adult hens. This made them believe that contact infection might result between mature birds and carriers, and that the infection might be passed through the eggs. Beach and Davis [1927] concluded that transmission through the egg was possible in the light of their studies on *S. gallinarum* in baby chicks and the ovarian lesions in adults, the latter being indistinguishable from those caused by *S. pullorum*. Egg transmission studies were made by Simms [1945], the organism being isolated from yolks of fertile eggs, dead embryos, ailing baby chicks and from the ovaries of reactor hens.

S. gallinarum closely resembles *S. pullorum* and it is difficult to differentiate them. Van Heelsbergen [1929], Manninger [1930], Miessner [1930], Haupt [1930], Wagner [1934] and other European workers have considered the two organisms identical. Smith [1915] and Smith and Ten Broek [1915] worked exhaustively on

the two organisms and, while recognising their *similarity as regards their antigenic structure,* noted the difference in the carbohydrate fermentation, such as gas production by *S. pullorum* and fermentation of maltose by *S. gallinarum*. Hadley *et al.* [1917] reported infection in adult fowl due to non-gas producing type of pullorum. The gas producing type has come to be known as *S. pullorum* '*A*' and non-gas producing type as *S. pullorum* '*B*'. Muslow [1919] found that certain strains of S. pullorum fermented maltose and hence suggested dulcitol as a better differentiating sugar. Hendrickson [1927] concurred with Muslow in using serum water as a basic medium for sugars. He attributed Maltose fermentation by *S. pullorum* to the materials and methods employed for the cultivation of the organism and suggested peptone water instead. He considered S. gallinarum as maltose, dulcite and dextrain positive. Van Roekel [1937] isolated strains of *S. pullorum* which fermented maltose within a few hours. Hinshaw [1941] showed by fermentation of carbohydrates that there were several sub-species in *S. pullorum* and *S. gallinarum* groups, like *S. pullorum* intermediate, type 'A', which produced acid and gas in maltose, dulcite and arabinose, and *S. pullorum* intermediate 'B' which produced acid only in maltose, dulcite and arabinose, but none of the two fermented xylose in addition, like *S. gallinarum*.

Experimental work

Epidemiology. In the beginning of 1950 an acute outbreak of fowl typhoid occurred in a large poultry farm. First the adults and later the baby chicks were affected perhaps in consequence of hatching from the carriers. This made a study of its various aspects possible for the first time in India. The disease spread rapidly into the majority of the breeding pens. The birds in full lay were found droopy ruffle feathered, off feed and reluctant to move. Some had matted fluff due to greenish diarrhoea which developed in birds that lingered on for a few days. The temperature was 1° to 3° F. above normal, comb and wattles became cyanotic and death supervened after a couple of days of ailment (Plate XI. fig. 1.)

The early cases were peracute for the first two weeks and later the rate of mortality decreased and the course also became protracted, the symptoms being pallor of the comb and wattels and profuse green diarrhoea. Pending diagnosis, the spread of the disease was checked by depopulation of the visibly affected birds and sanitation. The probability of food poisoning was eliminated by biological and cultural tests.

Post-mortem findings. Over a hundred birds were autopsied. No special lesions were noticed in peracute cases and the blood was negative under the microscope for organisms. In cases of delayed mortality a common though not constant lesions was a linear band of haemorrhage varying from 2 to 3 m.m. in width in the sub-mucosa of the proventriculus near the gizzard-end. Occasionally there were necrotic ulcers in the horny epithelium of the gizzard. Liver was enlarged and friable containing numerous yellow or grey necrotic foci, almost similar to the lesion seen in diffuse lymphomatosis. Pericardium showed serofibrinous adhesion and in the heart wall large necrotic foci as big as the seeds of garden peas were observed. The spleen was enlarged being more than thrice the normal size with numerous small areas of necrosis, or at times studied with multiple nodules. Acute cases showed a severe haemorrhagic follicular enteritis, while in more prolonged cases circular

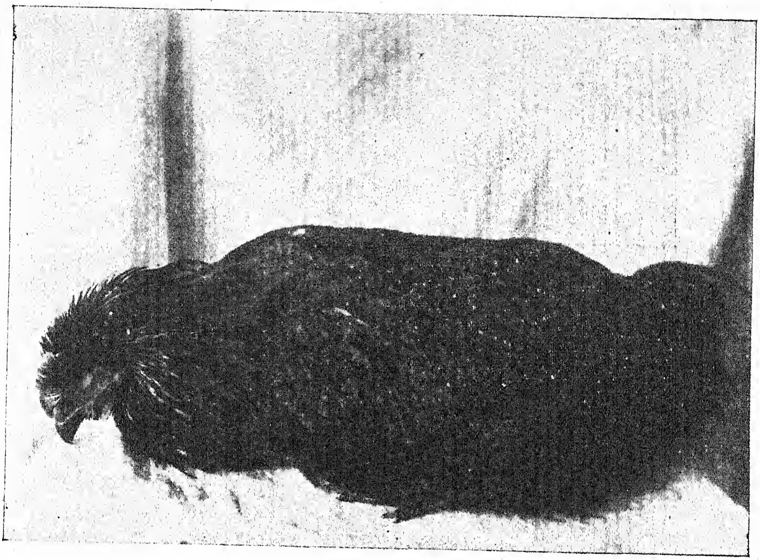

FIG. 1. A peracute case of Fowl typhoid.

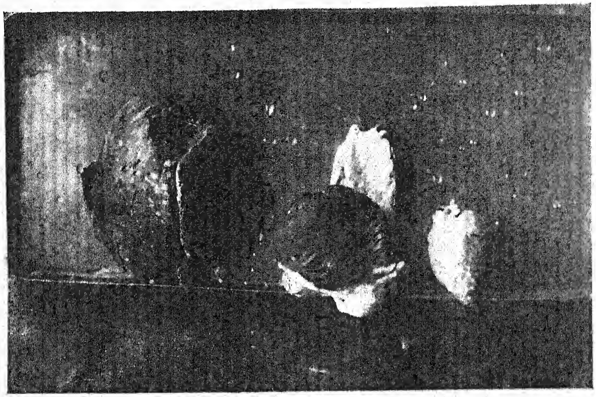

FIG. 2. Liver, gizzard, proventricular and heart lesions in adult fowl.

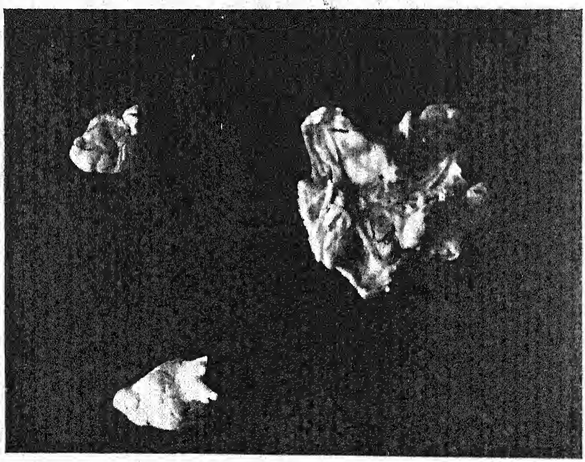

FIG. 3. Lung and heart lesions in baby chicks.

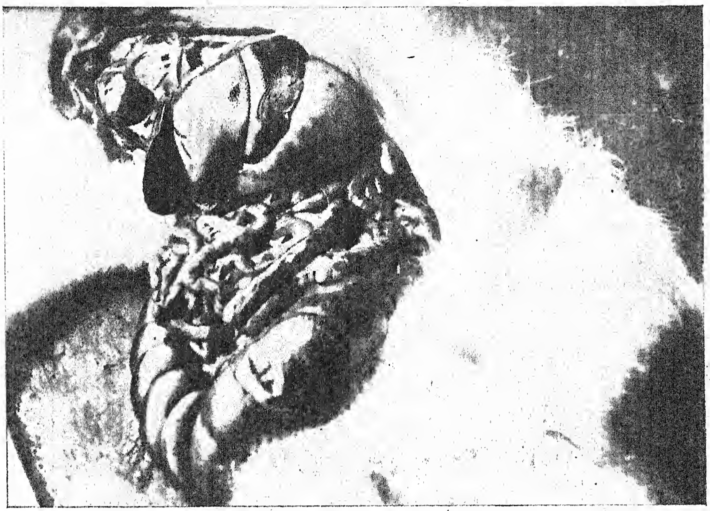

FIG. 4. Rabbit septicaemic lesions on heart, stomach and intestines.

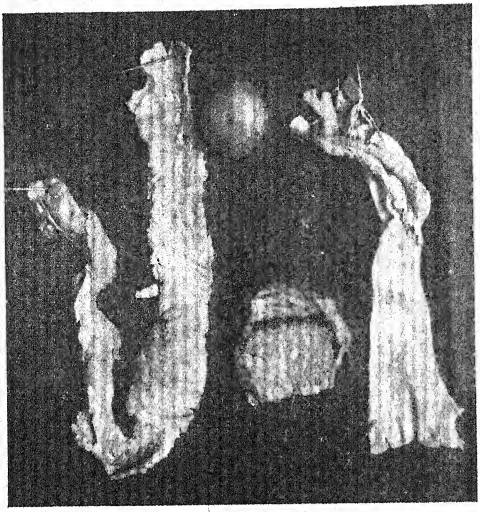

FIG. 5. Necrotic lesions of intestines, liancer haemorrhage in proventriculus, enlarged spleen, and follicular enteritis in the adult fowl.

ulcers were observed in the affected portions particularly near the terminal part of the intestines. The gall bladder was usually distended with very dark green bile and the same was noticed in the fluid evacuations. About the third week, involvement of the ovaries were noticed in a few cases. Most of the ova were healthy but one or two bluish or greenish angular ova could be noticed on minute examination. (Plate XII, fig. 2 and Plate XIV, fig. 5.)

After the cessation of mortality, cases of ruptured liver and yolk peritonitis were often seen in the *post-mortems* for over six months as a sequel to the disease. Such livers presented a mass of cicatrices of healed lesions with chalky deposits. After a removal of the reactors, from survivors of the flock, cases of yolk peritonitis decreased, but those of ruptured livers persisted. The liver cultures were however negative for Salmonella.

Histopathology. The average blood picture of adults in peracute cases showed the following counts :—

Erythrocytes	2,300,000 per c.mm.
Leucocytes	138,000 ,, ,,

The Leucocytes showed a heavy increase. In one rare instance the blood smear showed organisms.

Average differential count

Neutrophiles	66·7 per cent
Eosinophiles	1·9 per cent
Basophiles	0 per cent
Lymphocytes	24 per cent
Monocytes	7·4 per cent

The above figures, which are an average of several series of observations, show decrease of lymphocytes and increase of neutrophiles.

In subacute cases the anaemic changes were so profund that blood appeared watery. The heart tissue on examination showed disintegration of the muscle fibres and infiltration with mono-nuclear plasma and inflammatory cells. The liver showed disintegration of hepatic vessel walls and infiltration of tissue with inflammatory cells in acute and peracute cases, whereas in chronic cases fibreoblastic changes coupled with necrosed areas were evident.

In acute cases the lesions were not characteristic enough for a diagnosis independent of laboratory tests but later, enlargement of the spleen, lesions of the heart coupled with the absence of the organism in the blood suggested fowl typhoid.

Symptoms and lesions in baby chicks. A natural outbreak of the disease in baby chicks in the brooder house caused 21 to 63 per cent mortality in different pens, starting from the sixth day with a peak about the ninth day, and then decreased by the third week.

The mortality in the natural outbreak in adult birds ranged from 13 per cent in White Leghorn to 21 per cent in Rhode Island Red birds. The male birds suffered much less than the female of the same breed. In peracute and acute cases there

were no characteristic symptoms or lesions, though the organism could be regularly recovered from the liver and heart blood.

When the course was prolonged, the chicks were found to huddle around the hover, while a few isolated themselves showing marked dyspnoea and droopiness. Diarrhoea was not always present in chicks less than a week old, but older ones had greyish fluid evacuations and pasting of the vent.

In acute cases no lesions were seen, but in more protracted ones the liver showed multiple pin-point haemorrhages, green and yellow stripes of discolouration, or miliary necrotic foci in varying degree. Unabsorbed yolk with a greenish sheen, lesions of greyish white necrotic nodules on heart wall and distortion of its shape were also observed. In some, stripes of petichiae in the proventriculus at the gizzard-end, lung lesions ranging from a slight congestion to pneumonia and rare lesions of irregular necrosis, or scattered white nodules were found. Experimental infection assumed a peracute form and hence lesions were not observed. (Plate XII, fig. 3.)

Experiments with chronic carriers of Salmonella gallinarum

Twenty eight reactors were reared in isolation for four months for experimental observation. When autopsied there were four 'no lesion' cases, which were negative for cultural tests as well. Among the rest there were six (one cock and five hens) whose end titres ranged from 1 to 20 to 1 to 80. The remaining birds were all showing high titres up to 5,180 and were culturally positive. The cock bird showed healed heart and liver lesions, but was culturally negative.

Severe chronic ovarian lesions (Plates XV and XVI, figs. 6 and 7) were found in the last group, while the low reacting hens showed but one or two aberrated ova with abnormal yolk contents. Organism were however regularly recovered from them.

Summary of lesions in chronic carriers. The lesions which were sequel to ovarian dysfunction were typical of Salpingitis, as described by Bunyea [1926] caused by *S. pullorum*, and were seen in fowl typhoid as well.

Egg production of Reactors. The average egg production of the farm flock was over 60 per cent during February, March, April and May, but for the same period the reactors averaged 19·5 per cent during the first month but later the production dwindled down to zero in the course of a further three months, when they were destroyed for examination.

Incidence of the disease in hatches from carrier eggs. A pen of 24 reactor hens were mated to a reactor cock and 48 eggs from this pen were collected. The shells were disinfected in 5 per cent warm solution of dettol and dried, and placed in a small incubator and the resulting hatch was brooded in isolation.

The results of the incubation were as follows :—

Infertiles	14 or 71 per cent. fertility
Dead germs	9
Dead embryos	19
Number hatched out	6 or 17.6 per cent. Hatchability

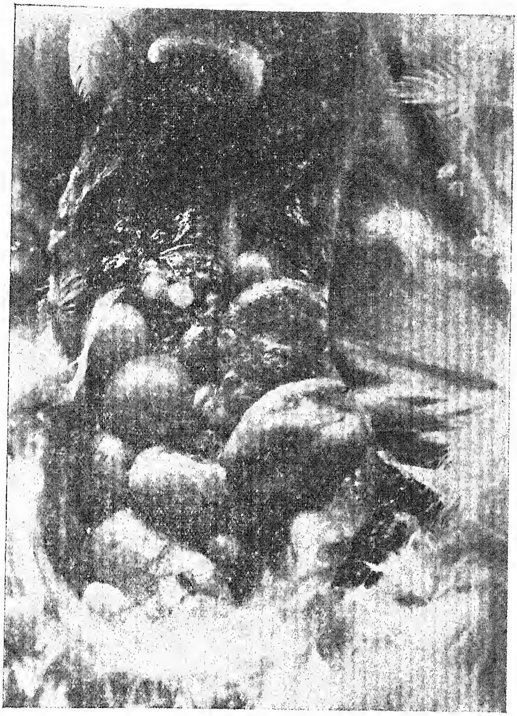

FIG. 6. Mis-shapen ova cystic ovary.

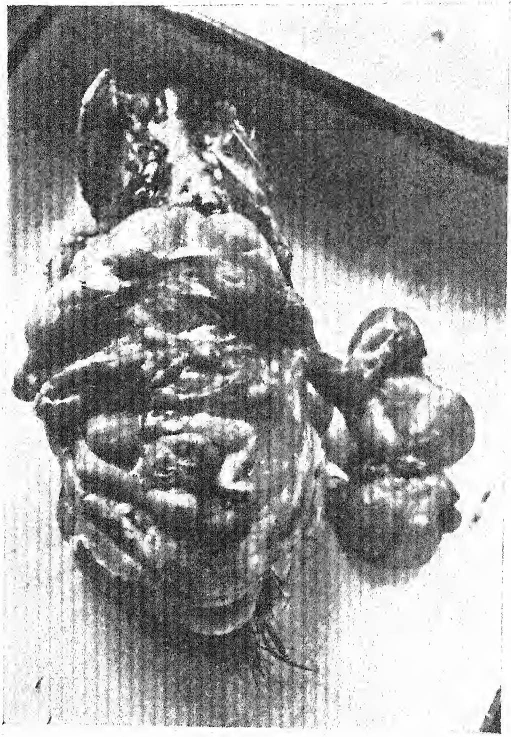

FIG. 7. Chronic yolk peritonitis and cystic ovary.

Four out of the six chicks were seropositive after 12 days but heart blood and liver cultures were sterile. The organism was recovered from the livers of the two chicks which were seronegative.

Bacteriology

Isolation of the organism from the heart blood and liver. The organism was isolated from acute cases from the heart blood and livers of chicks and adults dying after a brief period of illness. The colonies on plain agar appeared as fine granular pin-point in 24 hours and developed into smooth glistening translucent hemispheres in 48 hours of incubation, and later they varied from round to angular. The heart blood usually yielded a pure culture, while liver cultures sometimes required sieving through brilliant green broth and plating on to Macconkey media.

Isolation from the ovaries of the reactors. In cases of abnormal ovum, culture was obtained by searing the ovisac puncturing and pipetting out the whole contents into brilliant green broth, about 200 c.c. per ovum, and incubating for 48 hours and then plating on to Macconkey for recovery of the organism. Where lesions were not well marked and the ovary was found arrested in its growth, the bunch of the immature ova was tritutated and transferred to a large quantity of tetrathionate broth from 200 to 500 c.c. depending upon the size of the bunch and incubated for 24 to 48 hours before recovery by plating on to Macconkey. By these two methods 19 out of 24 ovaries of reactors yielded positive cultures.

Isolation of the organism from eggs of the Reactors. Thirty-six eggs from a pen exculsively of carriers were cultured in five batches. The eggs were opened after washing in 5 per cent dettol, dipping in alcohol, flaming lightly, and opening into a sterile petri dish. The whole yolk was spooned out into 200 c.c. of broth, and incubated for 48 hours, while the white was discarded. The organism was recovered on Macconkey plate. 13 out of the 36 eggs thus cultured gave positive results which proves that over 36 per cent of the eggs laid by carriers might be infected.

Isolation from the intestines of baby chicks and adults. Materials from the duodenum of baby chicks with white diarrhoea, and from adults showing enteritis were incubated in separate isolation experiments in terathionate broth to which brilliant green in 1 to 50,000 proportion had been added. After 48 hours of incubation the organism was recovered on Macconkey media in the usual way, but several sievings were continued through the special media before pure culture was obtained.

Indentification of S. gallinarum. In all cases of isolation on Macconkey plates fine white colonies were picked up and sown on agar slants serially. Those colonies which yielded non-motile gram negative slender rods were tested for their antigenic specificity by the rapid serum method of Stafseth and Carbutt [1940] before submitting to principal sugar tests for confirmation.

Pathogenicity. Rabbits and guinea pigs were killed in 48 hours by the subcutaneous route, evincing septicaemic lesions of an acute haemorrhagic type on heart, stomach, and intestines (Plate XIII, fig. 4). It proved fatal to pigeons after five days by the intramuscular route. The pigeon went down on both legs and was supporting itself on its out-spread wings on the fourth day. The *post-mortem* findings were, large areas of necrosis in the pectoral muscles at the site of inoculation

and acute arthritis of wing and limb joints. Both white and black rats were resistant. A mortality of 50 per cent was observed in adult fowls when infected by intra-muscular route, birds succumbing from 7 to 11 days. Three healthy bird kept successively in the same cage where previously sick birds had been confined became sero-positive in 11 to 15 days but the infection was sub-clinical. 18 hour broth culture of the organism in dilution corresponding to Brown's standard opacity tube 1, in 0·1 c.c. dose subcutaneously, proved *cent per cent* fatal in 3 to 5 days, to six one day old baby chicks. Oral dosing of the culture suspension in 0·5 c.c. dose was equally fatal in 5 to 8 days, in a day-old chick, with two survivors among the ten birds infected, which also became sero-positive after eleven days. Three incontact chicks with the above lot died of the disease in 7 to 10 days, organisms being recovered from them.

Oral dosing of the culture suspension described above, along with a 16 per cent sodium solution of sulphamezathine in drinking water at 0·2 per cent level of the active drug for one week in a lot of 20 day-old chicks failed to prevent mortality. 16 died within a week and two of the rest were sero-positive. Organism was recovered from all the dead chicks.

Morphology. The strain of the organism isolated from the adults produced typical disease in chicks and *vice-verse*.

The two strains were identical as regards their behaviour on routine laboratory media. They exhibited the following characters :—

Slender gram negative, non-motile rods with rounded ends.

Cultural characters :—

Agar colonies :—Moist greyish circular, entire, and dome shaped.

Agar slant :—Thin grey streak, moist with irregular margin.

Broth :—Turbid with heavy flocculent sediment.

Biochemistry. Litmus milk was rendered alkaline. V. P., M. R. and Indole tests were negative. Hydrogen sulphide was not formed. Carbohydrate fermentation reactions were put up with peptone water as a base and Andrades indicator to determine acid production as suggested by Bergey [1948].

The following sugars were fermented within 48 hours without gas production by both the adult and chick organisms :—glucose, mannite, maltose, dextrin, dulcite, mannose, laevulose, xylose, raffinose, rhamnose, arabinose, and galactose, except xylose which took 96 hours, and no changes were observed subsequently up to 14 days. Lactose, sucrose, glycerol, salicin, sorbitol, adonitol and inositol were not fermented. Six months later the tests were repeated and gave the same identical results.

Serology. The organism from the adult bird No. 399 was tested by the slow tube method against homologous serum collected from strongly reacting birds and the titres were found to be as high as 1 to 12,000. Among the chronic cases there were a small percentage of birds four months after the outbreak with end titres as low as 1 to 20, 1 to 40, and 1 to 80. Acute and subacute cases of the disease died before agglutinins could be demonstrated. Under experimental conditions agglutinins could only be perceived usually on the eleventh day in birds that were given killed-cultures for antiserum preparation.

Tube test using the antigen S.p.17A (a standard Hull Que strain of *Salmonella pullorum* received through the courtesy of the Dominion Animal Pathologist, Canada) was positive to complete titre with the natural serum from the reacting bird, similarly both chick no. 586 and adult no. 399 antigens reacted to complete titre with the immune serum prepared from stock culture S.p. 17A. The same serum S.p.17A absorbed with chick and adult antigens was found to be completely free of the homologous agglutinins, conversely the adult no. 399 serum absorbed with the homologous antigen failed to agglutinate S. p. 17A and chick antigens. Reciprocal absorption test using rabbit anti-serum prepared in the laboratory resulted in complete absorption of agglutinins for the no. 399 organism as well as the standard stock pullorum organism.

Discussion

The course and Post-mortem lesions.—The course and lesions in adult fowl and baby chick due to *S. gallinarum* are similar to those described by Bushnell [1948], but with minor difference like the presence of a band of haemorrhage in the submucosa of the proventriculus and cyanotic instead of anaemic comb and wattles in the adult. The latter has been described as a rare finding by Van Straaten and Te Hennepee [1918]. The classic bronze coloured liver ascribed to this disease as a diagnostic lesion was seen only once in over one hundred *post-mortems* performed.

Experiments with Reactor hens.—The results of hatching from reactor eggs showed a 82·3 per cent embryo mortality, and a 33 per cent infected hatch in extreme cases of a total carrier flock. Further, the organism was also recovered from 36 per cent of the carrier eggs in the isolation experiments. These findings are in close agreement with Simms' work [1946] in which he reports 37 per cent of hens from inoculated group and 27 per cent of birds recovering from a natural infection laid infected eggs.

Pathogenicitys—The organism proved equally pathogenic to baby chicks and adults under natural conditions, though the disease has been described as of the young adults. The reports about the susceptibility of pigeon to *S. gallinarum* is variable. Bushnell [1948] stated that guinea pigs were very resistant. Klein [1889] and Lucet [1891] were unable to infect pigeons even with heavy doses of the culture subcutaneously, while Moore [1895] and Pfeiler and Roepke [1917] proved that the pigeon was vulnerable. The organism under discussion is very fatal both to guinea-pigs and pigeons. Similarly, the susceptibility of rabbit is reported to be low by Pfeiler [1920], Hinshaw and Taylor [1933], while our organism has proved lethal to rabbits even in minute doses of 0·2 c.c. of a saline suspension with turbidity adjusted to Brown's standard opacity tube No. 1.

Sulphamezathine in Fowl Typhoid.—Sulphonomide drugs have been tried by several workers with conflicting results in Fowl typhoid [Bushnell, 1948], and the experiment under review showed that it is not of much value.

Classification of the organism.—The chick and the adult bird organisms are serologically identical with the standard *S. pullorum* strain of Hill Que, Canada. Both the non-motile organisms could be differentiated from *S. pullorum* type 'A' on the basis of not forming gas in sugars, and from the non-gas forming or type 'B' of pullorum by the quick fermentation of maltose, dulcite and dextrin [Hendrickson, 1927].

Both strains differ from the intermediate type 'B' of pullorum by the fermentation of xylose and dextrin and from intermediate type 'A' by the non production of gas as well as fermentation of xylose and dextrin. Therefore the chick and adult strains are identical and belong to the same species *S. gallinarum*. (Table I.)

TABLE I

Carbohydrate fermentation reactions

Sugars	Standard pullorum organism (S.p. 17A)	No. 586 Chick organisms	No. 399 Adult organism
Lactose	—	—	—
Sucrose	—	—	—
Glucose	+	+	+
Mannite	+	+	+
Maltose	—	+	+
Salicin	—	—	—
Dulcite	—	+	+
Mannose	+	+	+
Sorbitol	—	—	—
Laevulose	+	+	+
Dextrin	—	+	+
Raffinose	—	+	+
Rhamnose	+	+	+
Galactose	+	+	+
Xylose	+ (late)	+ (late)	+ (late)
Arabinose	+	+	+
Adonitol	—	—	—
Inositol	—	—	—

The disease was however completely eradicated by five repeated monthly tests with an improved rapid whole blood antigen [Rao 1950].

SUMMARY

The occurrence of an explosive outbreak of fowl typhoid, which took a heavy toll of the breeding stock on a large poultry farm, for the first time in India is described.

A virulent strain of the organism was isolated from eggs, baby-chicks and tissues of the adult fowl, and classified as *S. gallinarnm.* The outbreak was controlled and the disease was eradicated by blood testing of the stock.

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GEOGRAPHICAL DISTRIBUTION AND YEARLY AND SEASONAL INCIDENCE OF MAIN CONTAGIOUS DISEASES IN THE MADHYA PRADESH

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(With four text-figures)

ALL the available records relating to the main contagious diseases in Madhya Pradesh, with the exception of merged states for which no records could be had prior to 1950, were collected for the fifteen year period from 1936 to 1950 and analysed, and the yearly, seasonal, divisional and regional incidences as per rainfall and agricultural tracts respectively have been worked out.

Yearly Incidence

The total number of outbreaks in the different districts of the state were added up annually for the period mentioned above and are presented in tabular and graph forms below :—

Table I

Yearly incidence of the main contagious diseases.

—	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	
Rinderpost	1774	1793	1214	1995	1778	610	728	659	1045	1697	1791	409	216	1489	943	=18144
Haemorrhagic Septicaemia	1218	1382	1560	803	820	734	954	801	788	340	310	518	597	593	637	=12055
Blackquarter	196	1084	517	190	122	893	403	678	260	127	75	349	567	366	231	=5558
Anthrax	481	77	135	119	178	111	203	107	38	22	49	33	65	30	40	=1688
Foot and Mouth	4109	3449	4783	6394	5037	6262	5208	3505	3198	1413	3083	1792	2725	3657	1523	=56138

Table No. I and graph indicate a decline in the annual number of outbreaks of rinderpest, haemorrhagic septicaemia, anthrax and foot and mouth disease while there has been no significant change in the case of blackquarter. The marked reduction in the number of outbreaks of anthrax in 1937 as compared to the preceding year is attributable to more selective recording by the field staff of outbreaks under the head following special instructions to this effect.

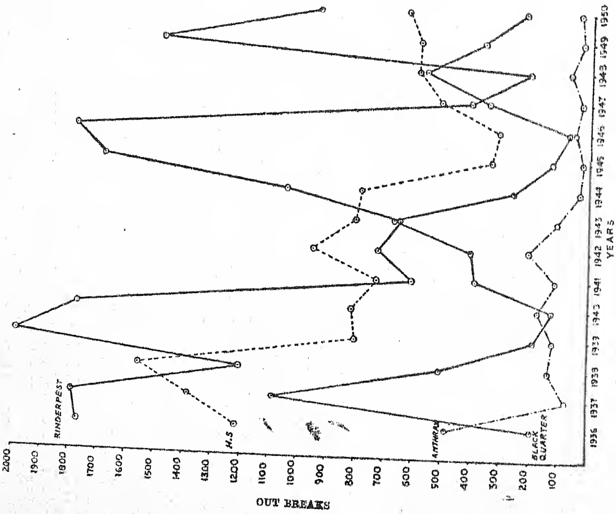

FIG. 14. Yearly incidence.

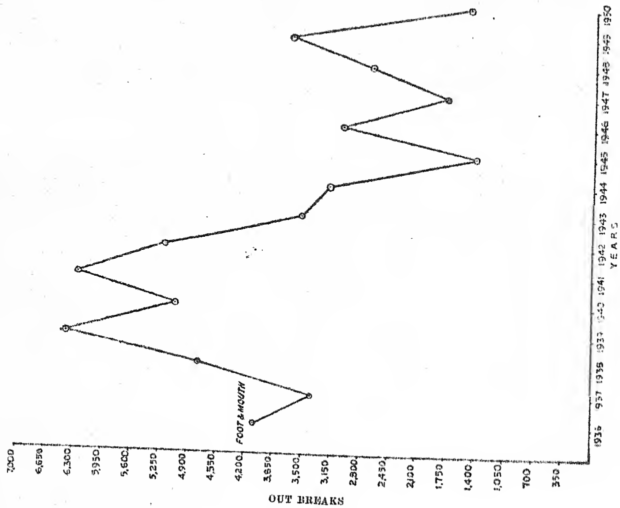

FIG. 15. Yearly incidence.

SEASONAL INCIDENCE

The outbreaks of all the diseases were compounded monthwise to determine the seasonal incidence :—

TABLE II

Outbreaks of diseases compounded monthwise

	January	February	March	April	May	June	July	August	September	October	November	December	
Rinderpest	1451	1476	1802	1765	1662	1272	1562	1699	1906	1384	1058	1104	=18141
Haemorrhagic Septicaemia.	155	232	243	400	478	1339	3336	3121	1779	597	236	139	=12055
Blackquarter	90	81	136	206	254	586	1790	1262	667	259	141	86	= 5558
Anthrax	62	63	97	159	190	250	300	224	167	91	41	44	= 1688
Foot and Mouth	8542	13367	13900	7094	2199	930	1382	1608	1453	1106	1481	3076	=56138

The largest number of outbreaks of rinderpest occur during the months of March and September while the disease is at comparatively low ebb in November and December. The peak period in haemorrhagic septicaemia coincides with the months of July and August and the disease then abates registering a low incidence during winter from November to February and again shows a significant increase from April onwards with a marked rise in June due to the onset of rains, and the position in respect of abatement and subsequent rise is similar to that of blackquarter and anthrax. In the case of blackquarter also the acme is attained in July and the largest number of outbreaks occur during July and August. With anthrax the peak period is contemporaneous with the rainy months from June to August and the acme again lies in July. Lastly, as regards ' foot and mouth ', the disease is at its peak during February and March and then shows considerable subsidence touching a very low level in June with a significant increase being registered from the month of December onwards. Calculations to show that the high incidence of contagious diseases during certain months referred to in particular above, as also the low incidence of rinderpest in November and December, can be held to be statistically significant are detailed below :

Statistical significance of the chance factor

Calculating on the basis of the formula given below the value of 't' in the different cases is specified under the respective heads :

$$t = \frac{(\overline{X1} - \overline{X2})}{S\overline{X}}$$

When $\overline{X1}$ =mean of sample 1,

$\overline{X2}$=mean of sample 2,

and $S\overline{X}$ =standard error.

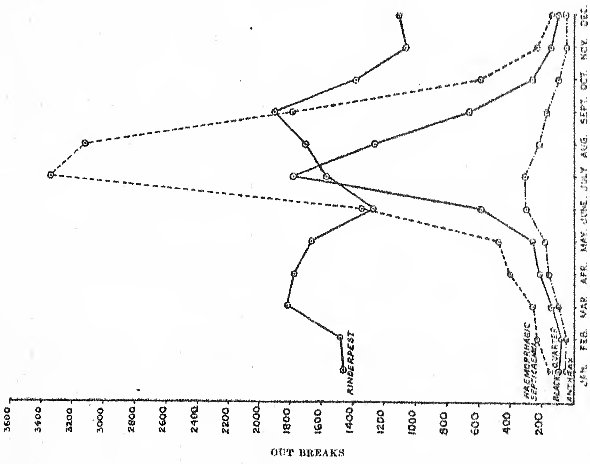

Fig. 2a. Seasonal Distribution.

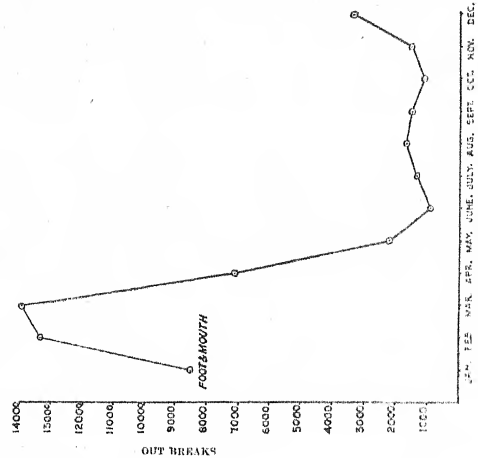

Fig. 2b. Seasonal Distribution.

1. *Rinderpest*—

(*a*) March and September (months of highest incidence) — t=2·3, significant at 5 per cent level.

(*b*) November and December (months of lowest incidence) — t=3·4, significant at 1 per cent level.

2. *Haemorrhagic septicaemia.*—

July and August (months of highest incidence). — t=6·5, significant at 0·1 per cent level.

3. *Blackquarter*—

July and August (months of highest incidence) — t=7·1, significant at 0·1 per cent level.

4. *Anthrax*—

June, July and August (months of highest incidence) — t=3·7, significant at 1 per cent level.

5. *Foot and mouth disease*—

February and March (months of highest incidence). — t=5·4 significant at 0·1 per cent level.

Divisional Distribution of Outbreaks

The respective number of outbreaks of each disease in the four divisions of the state were found out by adding up the annual district totals for the years 1936 to 1950 and the number of outbreaks per 10,000 animals and 100 sq. miles area during this period were worked out to get a comparative idea of the incidence. Calculations were also made on the basis of the population and area of the whole of the state and it was ascertained that there were 68·4 outbreaks per 10,000 animals and 96·1 outbreaks per 100 sq. miles giving annual averages of 4·5 and 6·4 outbreaks respectively. Table III and map below show the divisional distribution of outbreaks.

TABLE III

Divisional distribution of the number of outbreaks

Name of division	Cattle population	Area square miles	Rinderpest			Haemorrhagic septicaemia			Blackquarter			Anthrax			Foot and Mouth		
			Total outbreaks	Per 10,000 bovines	Per 100 Sq. miles	Total outbreaks	Per 10,000 bovines	Per 100 Sq. miles	Total outbreaks	Per 10,000 bovines	Per 100 Sq. miles	Total outbreaks	Per 10,000 bovines	Per 100 Sq. miles	Total outbreaks	Per 10,000 bovines	Per 100 Sq. miles
Jabalpur division	3517445	25638	4603	13·4	17·9	2393	6·8	9·2	1683	4·7	6·5	366	1·0	1·4	12282	34·9	47·8
Nagpur division	3237444	27284	4259	13·1	15·5	2942	9·1	10·7	2665	8·2	9·7	469	1·44	1·71	13681	42·2	50·1
Berar division	2225186	17767	1977	8·8	11·1	3383	15·2	19·0	898	4·3	5·05	166	0·74	0·93	9538	42·8	53·6
Chhattisgarh division.	4637131	27719	7302	15·7	26·3	3337	7·2	12·0	312	0·66	1·12	687	1·4	2·4	20637	44·5	74·4

Both rinderpest and foot and mouth diseases are more common in Chhattisgarh division due probably to it being a rice growing calcium deficient area where cattle do not thrive thus leading to regular import of cattle from outside for replacement with consequently more chance of dissemination of infection, and, also, in part, to the greater relative density of cattle population in the division. Haemorrhagic septicaemia occurs more in Berar where the rainfall is on the whole low (32 in.–34 in.) as compared to other places. Blackquarter is commonest in the Nagpur division and the disease is of little consequence in Chhattisgarh. The incidence of anthrax is comparatively low all over the province though more outbreaks occur in the Chhattisgarh and Nagpur divisions.

Incidence of Diseases According to Rainfall

The Madhya Pradesh can be divided from the point of view of rainfall into three regions :—(1) 32 to 34 inches ; (ii) 42 to 52 inches and (iii) 55 to 65 inches. The respective number of outbreaks of different diseases per 10,000 bovines and 100 sq. miles area, during the period under review in the regions, are given below.

Table IV

Incidence of diseases according to rainfall

OUTBREAKS

Region	Rinder pest		Haemorrhagic septicaemia		Blackquarter		Anthrax		Foot and Mouth.	
	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles
Region I (4 Districts (32 in. to 34 in.)	10·3	11·9	15·4	17·9	3·7	4·3	0·80	0·93	49·5	57·5
Region II (11 Districts) (42 in. to 52 in.)	13·8	19·8	7·5	10·8	4·4	6·3	1·1	1·6	41·7	56·9
Region III (4 Districts) (55 in. to 65 in.)	13·35	13·15	8·0	11·4	2·7	4·0	1·8	2·6	30·3	43·5

Rinderpest is less common in region No. 1 (32 in.—34 in.) haemorrhagic septicaemia is, however, more common here as compared to the other two regions. Outbreaks of blackquarter occur more in region No. 2 (42 in. to 52 in.). The incidence of anthrax is highest in region No. 3 (55 in. to 65 in.) while that of foot and mouth disease in this area is on the other hand the lowest of the lot.

Distribution of Diseases According to Agricultural Zones

The Madhya Pradesh can be divided into three tracts on the basis of the princi pal crops grown which again is dependent on the nature of soil etc., in the respective areas *viz.*, (*a*) cotton tract, (*b*) wheat tract, and (*c*) rice tract.

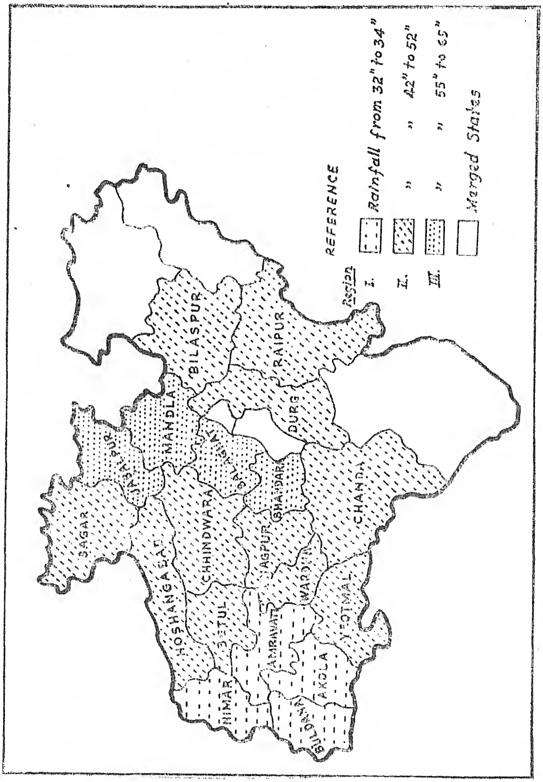

FIG. 4. Regional distribution of rainfall in the Madhya Pradesh (excluding merged states).

Table V

Incidence of diseases according to Agricultural zones

Outbreaks

Agricultural zones	Rinderpest		Haemorrhagic septicaemia		Blackquarter		Anthrax		Foot and Mouth	
	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles	Per 10,000 bovines	Per 100 Sq. miles
1. Cotton tract (7 Districts).	10·4	14·0	13·1	16·1	5·0	6·3	1·2	1·6	47·0	59·0
2. Wheat tract (6 Districts).	12·2	18·0	7·5	11·07	7·1	10·5	0·8	1·2	32·6	15·9
3. Rice tract (6 Districts).	15·7	22·8	6·9	10·0	0·76	1·1	1·5	2·2	41·2	61·5

Rinderpest and anthrax are more common in the rice tract, haemorrhagic septicaemia in the cotton tract, blackquarter in the wheat tract, and foot and mouth disease in the cotton and rice tracts. The incidence of blackquarter is very low in the rice tract.

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AN IMPROVED *SALMONELLA ANTIGEN* FOR THE RAPID WHOLE BLOOD AGGLUTINATION TEST

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THE occurrence of *Salmonella pullorum/gallinarum*, *S. aertrycke*, and certain members of other paratyphoid groups in commercial poultry flocks in widely separated areas since 1949 for the first time in this country, has created a serious hazard to the growing poultry industry, and a potential menace to public health, and has made the study [Rao *et al.*, 1950] of the various aspects of the disease possible and its control imperative.

Fifty-two strains of Salmonella pathogenic to man have been so far isolated in the U. S. A. from dried eggs, chickens and turkeys [Soloway *et al.*, 1947]. Even *S. pullorum*, which was considered host-specific to chickens only, has been incriminated in several cases of human food poisoning. [Mitchell, Garlock and Brokkahn, 1946 and Judefind, 1947.]

The control plans of *Salmonella pullorum/gallinarum* primarily aim at the elimination of the infected bird by any one of the accepted blood test methods, viz. the slow tube, the rapid serum, or the whole blood agglutination test. On the contrary, paratyphoid (motile) organism, usually not being yolk borne, does not seem to create such a serious 'carrier' problem as its spread is by shell contamination or by penetration of the shell during incubation [Schalm, 1937, and Wilson, 1944]. The yolk-borne nature of *S. typhimurium* infection recorded by Wilson [1949] is considered as exceptional and rare, the motile organism as a rule being localized in the intestine and occasionally in the gall bladder [Buxton and Gordon, 1947]. The egg-shells become infected by contaminated faeces. Blood tests are found useless as agglutinin production by the carrier birds is erratic and therefore unreliable for diagnostic purpose. The disease, however, is controlled for all practical purposes by incubator fumigation and farm sanitation [Buxton, 1948]. In rare cases where direct yolk transmission is proved it would be necessary to test each bird with 'O' and 'H' antigen from the specific organism by the slow tube method, combined with incubator fumigation [Dobson, 1950]. Such special tests are not carried out as a routine by most laboratories because of the complexities of the antigenic structure involved and the difficulty of obtaining a satisfactory antigen which will produce reliable agglutination results [Van Roekel, 1950].

As for *S. pullorum/gallinarum* infection, whole blood rapid plate agglutination test or simply 'spot test' is perhaps the only method capable of easy application in the field, and hence much work has been done to improve the specificity and the keeping qualities of the antigen. For instance, an alcoholised antigen, issued by the Poultry Diagnosis Laboratory Department, Weybridge, has been prepared from

a single strain of *S. pullorum* containing the full complement of XII^2 factor, variants being few in the prevailing strains examined in England. An antigen, containing three standard and two yonie (variants) strains, is used in Canada as the prevalence of the three components of XII factor [Edwards and Burner, 1946] and their quantitative Variants especially of XII^2 amongst the strain's isolated has been recognised [Gwatkin, 1945 and Gwatkin and Bond, 1945]. Lately, an effort is being made to incorporate a strain or strains of *S. pullorum* containing both XII^2 and XII^3 antigenic components [Van Roekal, 1950]. Experimental work has also been directed to increase the agglutinability and secure a higher yield of the organism on special media like the Veal infusion agar enriched with glycerine and sulphur [Macdonald 'K' antigen, 1946], or glycerine and sodium-thiosulphate as used by Weybridge Poultry Department Laboratories [Dobson, 1950].

A review of literature since Bunyea, Hall and Dorset [1929] applied the whole blood test for detection of pullorum carriers, reveals a diversity of views both as regards the method and accuracy though the trend of research has been towards increasing the efficiency by improving the specificity, agglutinability and keeping qualities of the antigen.

Edwards and Hull [1929] have reported that even slide serum tests were not satisfactory in birds whose end titres were lower than 1 to 80. In most laboratories the diagnostic titre is 1 to 40; and in some 1 to 20 for the hen and 1 to 10 for the cock [Gaiger and Davis, 1946].

Lubbehosen and Beach [1935] and Hinshaw, Harr and Niemeyer [1940] considered the test was not efficient enough to be used exclusively, while Stafseth [1938], Reid [1938] Barger and Torry [1933] and Winter [1939] recorded goods correlation between the tube and plate tests.

Linn, Leask, Gunderson and Slack [1944] concluded that the routine standard 'spot' antigen then in vogue in the U. S. A. would detect titres as low as 1 to 10 in the majority of cases and was superior to Macdonald's 'K' antigen [1941], grown on special media for detecting low titre birds. Gordon [1947] has recorded very close agreement between the whole blood spot and the tube tests with the alcoholic antigen now in use in England.

Diverse views and methods of the workers show that antigens vary in sensitivity; yet when a suitable one is used the results closely agree with the tube tests. To obtain further information on the subject it was found necessary to compare and select, from the available information, the one that could make *finer* distinctions in the reading of doubtful reactions, and adapt it for use so that there might be uniformity in testing birds all over this country.

EXPERIMENTAL

Material and Methods. As a preliminary work the following antigens were selected for comparison with the one locally produced, herein called Experimental No. 3.

1. *Antigen of Schaffer et al. [1931].*

Culture media. Beef infusion agar pH 7·2 incubated for 72 hours at 37·5°C. Suspending fluid 1 per cent formaline (0·4 per cent actual formaldehyde in 0·85 per cent sodium chloride solution) *Dye used.* Crystal violet 1 per cent solution, 3 c.c. per 100 c.c. of antigen.

2. *Antigen of Coburn and Stafseth* [*1931*].

Culture media. Beef and chicken infusion agar pH 7·2 incubated for 72 hours at 37·5° C. Suspending fluid—Phenol 0·5 per cent in N.S.S. *Dye used.* Saturated alcoholic solution of gentian violet 1 c.c. and brilliant green 5 c.c. of 0·1 per cent solution for every 100 c.c. of antigen.

3. *Experimental antigen No. 3.*

Glucose glycerine agar for dissociation plates for colony selection, before charging the Special solid media.

2. Beef infusion agar enriched with glycerine and sodium thiosulphate for roux flask. pH of media 7·0 and incubated for 72 hours at 37·5°C.

Suspending Fluid. 12 per cent sodium chloride solution containing 0·125 formaldehyde (40 per cent) and 0·125 phenol per 100 c.c. of the solution. The pH of final antigen at the time of test is 8·0 by addition of one per cent sodium-hydroxide (a final pH of 6·5 has also given good results).

Dye used. Crystal violet 0·03 per cent solution, 3 c.c. per 100 c.c. of the antigen. Except for the variations as regards media, buffer solutions, dye and pH with respect to different antigens the methods of washing, collection, preservation and rendering the product safe for use on poultry farms, were same for the three antigens under study.

A single strain of *S. gallinarum* No. 399 was used to prepare the antigens. This strain was highly specific and had been isolated recently from a virulent outbreak, and it was being used to eliminate reactor. The organism was found to have a good antigenic property as revealed by cross agglutination and absorption tests with the standard *S. pullorum* stock strain, obtained from Hull, Que, through the courtesy of the Dominion Animal Pathologist, Canada. The organism was plated out several times and individual smooth colonies picked out after 48 hours incubation of the plates at 37·5° C., under low power of the microscope. Each colony was seeded into a peptone broth, and those showing even suspension of growth following 24 hours incubation at 37·5° C. without sedimentation were selected for antigen preparation. 1½ c.c. of the culture was flowed over the surface of the solid media in each roux flask uniformly. The flasks were paraffin sealed and incubated for 72 hours at 37·5° C., and those that showed a dense uniform growth were washed with 10 c.c. of their respective Suspending fluids, by gently rocking for five minutes. The suspension was filtered through sterile absorbent cotton wool into coloured sterile stoppered bottles and preserved in darkness at 30°F. for a week or more until the organism was killed as proved by safety test. Dyes were added and density adjusted 100 times Brown's opacity tube No. 1 with their respective Suspending fluids shortly before use.

A pen of 28 carrier birds raised for four months in isolation was tested simultaneously with the above listed three antigens weather and temperature remaining constant during the period.

The details of the test are given in the table.

The low titre birds Nos. 1168, 237, 68, 7082, 1553 and 458 which were negativ to the first two antigens but positive to the third experimental Nos. 3 were bled fo

serum by venous puncture. These had been picked up previously from the flock in the fourth monthly test by the same antigen which was then under trial.

TABLE I

End titre of serum of birds which were exclusively positive to the experimental antigen No. 3

No. of birds	1168	1553	237	68	7082	458
End titre	1:60	1:47	1:40	1:16	1:32	1:20

Comparison by the standard tube agglutination was made as a check by a *single tube* of 1 to 80 dilution in all cases (Table I) and also in different lower dilutions in the above cited special cases of six birds.

The close agreement in low dilutions with the tube tests indicates the high antigenic specificity of the experimental antigen No. 3. The results were further checked by autopsy of the reactors and cultural tests, and these were again found to be mostly in agreement with the tube tests with the exception of four birds in which there were no discernable lesions and the cultural tests also proved negative.

The experimental antigen No. 3 when stored in darkness at 60° F. under refrigeration was found to be efficient up to 145 days.

The rapidity of agglutination was influenced by temperature up to 40° C. beyond which it did not hasten the reactions. Below 20° C. the agglutination was retarded.

Mechanical features of the test

Glass slides 2 in. × 3 in. were used in place of glass plates as these could be easily picked up after stirring, and rocked a few times to hasten reaction.

Finely drawn pipettes were employed for the delivery of a droplet of antigen to ensure constancy in measurements.

A thin glass rod was used to wipe off a constant quantity of blood from the snipped end of a serration of a comb. The blood droplet was about half the size of the antigen drop.

DISCUSSION

The experimental antigen No. 3 proved to be more sensitive than the other two antigens, as it detected low titre birds with titres ranging from 1 : 16 to 1 : 60. These low titre ones have subsequently been confirmed to be infected, by post-mortem findings as well as by cultural tests.

Following factors in the preparation of the antigen might have combinedly contributed towards its efficiency :

(1). Special medium, which produces a higher yield of organism with greater antigenic specificity, (2). 12 per cent hypertonic saline Suspending fluid which hastens the flocculation, by reducing the electric potential of the antigen, (3). The final *p*H of the antigen before use adjusted to 6·5 or 8·0 which makes it more agglutinable, and (4). Phenol and formaline used in low concentration of 0·125 per cent, as with

TABLE II

Recreations of Carrier fowls to the antigens, cultural tests, and autopsy lesions

Serial number	Bird No.	Antigen No. 1	Antigen No. 2	Exptl. antigen No. 3	Single tube test 1 to 80	End titre	Ovarian culture	Autopsy lesions
1	1147	+ + +	+ + +	+ + +	+	Not put up	+	Sacculated leathery cystic ovary.
2	1445	+ +	+ +	+ + +	+	"	+	Chronic yolk peritonitis.
3	7094	+ + +	+ + +	+ + +	+	"	+	Abnormal dark green ova.
4	1455	+ + +	+ + +	+ + +	+	+	+	Necrotic foci in both testis.
5	7082	—	—	+ +	—	1 : 32	Not done	Bird lost.
6	1132	+ +	+	+ +	+	Not put up	+	Chronic yolk peritonitis.
7	Nil	+ + +	+ + +	+ + +	+	"	+	Two green ova and pericarditis.
8	1168	—	—	+ + +	—	1 : 60	+	Single dark green ovum, healed liver lesion.
9	1447	+ + +	+ + +	+ + +	+	Not put up	+	Cystic ovary.
10	1528	+ +	+	+ +	+	"	—	Ovary appeared normal.
11	1632	+ +	+	+ +	+	"	+	Chronic yolk peritonitis.
12	68	—	—	+ +	—	1 : 48	+	To dark green ova.
13	1133	+ +	+ +	+ + +	+	Not put up	+	Chronic yolk peritonitis and heart lesions.
14	1713	+ +	+ +	+ + +	+	"	+	Chronic yolk peritonitis
15	1288	+ +	+ +	+ + +	+	"	+	Mis-shapen abnormal ova.
16	1745	+ +	+ +	+ +	+	"	—	Ovary appeared normal.

TABLE II—*contd.*

Recreations of Carrier fowls to the antigens cultural tests and autopsy lesions

Serial number.	Bird No.	Antigen No. 1	Antign No. 2	Exptl. antigen No. 3	Single tube test 1 to 80	End titre.	Ovarian culture	Autopsy lesions
17	1126	+ +	+	+ +	+	Not put up	—	Testis normal, liver lesions cicatrices.
18	1522	+ +	+	+ + +	+	„	+	Yolk peritonitis.
19	1159	+ +	+ +	+ + +	+	„	+	Mis-shapen ova.
20	7690	+ +	+ +	+ + +	+	„	+	Mis-shapen and green ova.
21	237	—	—	+	—	1 : 16	+	Arrested ovary.
22	1555	+ + +	+ + +	+ + +	+	Not put up	+	Yolk peritonitis.
23	1149	+ + +	+ +	+ + +	+	„	+	4 Mis-shapen ova.
24	295	+ + +	+ +	+ + +	+	„	Not done	Bird lost.
25	1419	+ + +	+ +	+ + +	+	„	+	2 Cystic ova.
26	1435	+ + +	+ +	+ + +	+	„	+	Mis-shapen abnormal ova.
27	1553	—	—	+ +	—	1 : 40	+	2 dark green small arrested ova.
28	458	—	—	+	—	1 : 20	—	Testis normal. Heart extensive adhesions of pericardium Cleatrices of liver lesions. This cock was kept under observation for six months. After it was picked up as reactor. It showed a decline in titre after the third monthly test, and was negative to both plate and tube test in the fourth test.

Grading of the various degree of reactions

(1) + + + Clumping of the antigen in well developed flocculi surrounded by a clear space, and clumps floating in clear fluid

(2) + + Weaker reaction consisting of small clearly visible clumps of antigen partially surrounded by a clear space

(3) + Just visible clumps to the naked eye and confirmed easily by a lens, (x 10)

a concentration of 0·2 to 0·3 per cent and *p*H adjusted to 8·0 or 8·2 the flocculent precipitate is prevented [Caseman George and Rettger, 1930].

The reaction graded No. 3 may be termed 'sandy' which is classified by some workers as non-specific, but in the opinion of the author is a result of weak agglutinin level in the blood, since the infection has been confirmed subsequently by post-mortem findings as well as cultural tests. Such a reaction has also been considered in an earlier work as indicating either an old quiescent infection or a very recent one by Linn. *et al.* [1944].

Summary

An improved antigen for *Salmonella pullorum/gallinarum* with a high antigenic specificity has been developed for use in the rapid whole blood agglutination plate test. It consists of a single strain of the organism colony selected from glucose glycerine agar plate, and grown on beef infusion agar containing sodium thiosulphate and glycerine, and suspended in 12 per cent sodium chloride solution, with 0·125 per cent formaline and 0·125 per cent phenol, killed and adjusted to a turbidity of 100 times Brown's opacity standard tube No. 1, and stained with addition of 0·03 per cent crystal violet and a final *p*H of 6·5 or 8·0 just before use.

This antigen proved superior to the two earlier ones, by detecting, carrier birds particularly of low titres ranging from 1 to 16 to 1 to 60.

The antigen has its qualities preserved up to 145 days under 60° F. refrigeration.

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CALOTROPIS-STANDARDISATION AND BIOLOGICAL ASSAY

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(With Plates XVII to XX)

CALOTROPIS GIGANTEA, commonly known by the following names in various Indian languages, *arka* (Sanskrit); *madar* (Hindi); *akand* (Bengali); *erukku* (Tamil); and *jelledu* (Telugu); has been dealt with in detail in most of the indigenous medical literature and is highly spoken of as a very good alterative and tonic, [Watt, 1893 and Chopra 1933]. A preliminary study made by the senior author and his colleagues on the various parts of the plant namely, the leaves, the flowers and the root bark, revealed that the leaf powder in small repeated doses exerted a tonic effect on the ruminants. Active principles were isolated from the leaves and the milky juice (*madar* juice) and they were found to be identical in their physiological activities, except that the one obtained from the latter was more potent. Both of them produced a sustained rise of blood pressure when given intravenously to anaesthetised dogs [Rathnasabapathy, Lakshman, Krishnaswamy and David, 1949].

Pitchandi in 1948, isolated the active principle of the *madar* juice in a pure crystalline form and proposed the formula $C_{24}H_{36}O_9$ provisionally and suggested the name 'Gigantin' to the substance thus obtained.

The pharmacology of gigantin has been thoroughly investigated by the authors both on the amphibians and the mammals and its action on all the organs with special reference to the cardio-vascular system has been worked out in detail. It was shown that the drug, by a direct action on the musculature of the heart and blood vessels produces a persistent rise of blood pressure; the efficiency of the heart is increased in physiological doses and that the action on the heart is similar to that of digitalis [Rathnasabapathy, David and Iswariah 1951].

With all these beneficial actions of a good drug, gigantin has its disadvantages as a good pharmaceutical preparation. As it is insoluble in water it is unsuitable for easy parenteral administration and as the quantity obtained is not commensurate with the labour and cost involved in its preparation, it is uneconomical for manufacture on a large scale. It was therefore considered profitable to utilise the leaves of *Calotropis gigantea*, which have got active principles having the same physiological activity as gigantin, in the form of tincture after standardisation. The close resemblance of calotropis and digitalis in their biological activities, suggested that tincture calotropis can be assayed in the same manner as tincture digitalis.

METHODS

Standardi-ation. The first standard prepared by the authors consists of a mixture of ten different samples of the leaves of *Calotropis gigantea*, collected from the city of Madras and its neighbourhood, dried at the room temperature of about 28°C. for about ten days, powdered to pass through a No. 60 sieve, dried in an electric hot air oven between 55°C. and 60°C. for six hours, ampouled in sterile containers and stored in the frigidaire. The moisture content of the standard powder was determined and found to be 2·45 per cent.

Unit. The unit of the first standard was defined as the acitvity contained in 0·1 gm. of the standard calotropis leaf powder prepared and kept in this laboratory, (The Department of Physiology, Madras Veterinary College, Madras.)

Biological Assay. The standard is now available for any one to compare an unknown sample of leaf to see how far this sample differed in acitvity from that of the standard. Among the various methods of comparison available namely the frog, guinea pig and cat methods, the cat method as used for the Third International Digitalis Standard by the World Health Organisation (7) was tried in this laboratory and found to be very satisfactory. In this method, the mean of the lethal dose of the sample being tested for a series of cats, expressed per Kg. body weight was compared with the mean obtained for the standard powder.

In brief the procedure adopted was as follows :—

Preparation and storage of tincture. The contents of the ampoule of the standard were weighed to the nearest milligram and transferred to a conical flask of 50 ml. capacity. For each gram of the powder 10 ml. of 80 per cent (by volume) alcohol was added. The stopper was inserted and the mixture shaken for 24 hours at room temperature (28°C.-30°C.) by mechanical means, which continuously brings the solid material into fresh contact with the liquid phase. Immediately afterwards it was filtered by pressure through the filter paper taking all possible precautions to avoid evaporation of the solvent. The tincture was then transferred to a hard, amber coloured glass bottle with tight closure and kept in the frigidaire and used within 30 days.

Preparation of diluted tincture. At the time of testing the standard tincture was diluted with 0·9 per cent sodium chloride to make 1 : 20. It was prepared fresh on the day of use and shaken before it is poured into the burette.

Selection of animal. Domestic cats free from gross evidence of disease and weighing between 2 to 4 Kg. were chosen for the assay. Weight was determined to the nearest 0·05 Kg. Those which upon examination appeared either obest, ematiated, lactating or pregnant were not used. The cats were starved for 16 hours before use.

The cats were anaesthetised with chloralose at the rate of 110 mg. per Kg. given intramuscularly dissolved in alcohol, two hours before use. The tincture was then injected through the femoral vein from a burette and artificial respiration through the tracheal canula was maintained throughout to ensure that the death was not due to respiratory failure. Carotid blood pressure was recorded kymographically.

TABLE I

Cat Assay of Standard Tincture Calotropis

Serial number	Date of experiment	Description of cat. Weight in Kg.	Initial B.P. mm. of Hg.	Amount of Tr. in c.c. to arrest the heart	Time taken for arrest of heart, in minutes	Average per Kg. in c.c.	Mean average	Standard deviation 'd'	Standard deviation of mean or standard error
1	29-11-50	F 2·15	90	27	27	12·56	13·48	—0·92	$\sqrt{\frac{(\Sigma d2)}{n(n-1)}}$
2	30-11-50	M 2·10	110	30	36	14·29		+0·81	Σ=Sum of. d=deviation.
3	4-12-50	F 2·00	90	26	25	13·00		—0·48	n=number of cats used.
4	4-12-50	M 2·08	55	25	25	12·32		—1·16	
5	6-12-50	M 2·08	60	29·3	27	14·09		+0·61	
6	1-12-50	F 2·05	90	25	25	12·19		—1·29	
7	8-12-50	M 2·35	110	37	37	15·74		+2·26	13·48±0·5619
8	9-12-50	M 2·05	70	26·5	26·5	12·93		—0·55	
9	12-12-50	M 2·55	100	46	45	18·04		+4·56	
10	12-12 50	M 2·25	90	32	33	14·22		+0·74	
11	14-12-50	F 2·05	80	19	19	9·27		—4·21	
12	18-12-50	M 2·45	70	27	24	11·02		—2·46	
13	18-12-50	M 2·40	70	32·5	31	13·54		+0·06	
14	19-12-50	M 2·00	130	31	31	15·50		+2·02	

F—Female; M—Male.

The tincture was injected as a continuous flow from the burette at the rate of about 1 c.c. per minute until the heart stops beating as indicated by the blood pressure record falling to the zero line. The time was noted at the beginning when the injection was started and when the end point was reached by a stop watch. Similarly the quantity of solution which was necessary to kill the cat was measured from the burette. A typical graph in the cat assay is shown in Plate XVII.

A total of 14 cats were used in the assay of standard and the mean of the results as calculated was $13{\cdot}48 \pm 0{\cdot}5619$ (Table I).

It is suggested that any sample to be tested should be treated in the same way and the mean of the results of not less than six cats should be determined for comparison with the standard.

The potency of the sample being tested, expressed in relation to that of the standard preparation, is determined by dividing the figure for the average lethal dose of the standard preparation by the figure for the average lethal dose of the sample being tested. It may also be expressed in per cent of the standard by multiplying the potency by hundred.

Pharmacology of Tincture Calotropis. The actions of *Tincture calotropis* are very similar to that of gigantin. 0·05 to 0·1 c.c. per Kg. body-weight of the tincture produces a sustained rise of blood pressure when given intra-venously to anaesthetised dogs, even after sympatholysis by ergotoxine and paralysis of the vagal terminals by atropinisation (Plate XIX). The volume of the spleen decreased considerably corresponding to the rise of blood pressure. Myocardiogram experiments in mammals showed that the systolic level of the ventricles and the auricles were considerably increased with the diastole being normal or slightly deficient, consequently the output per beat must have been more (Plate XVIII).

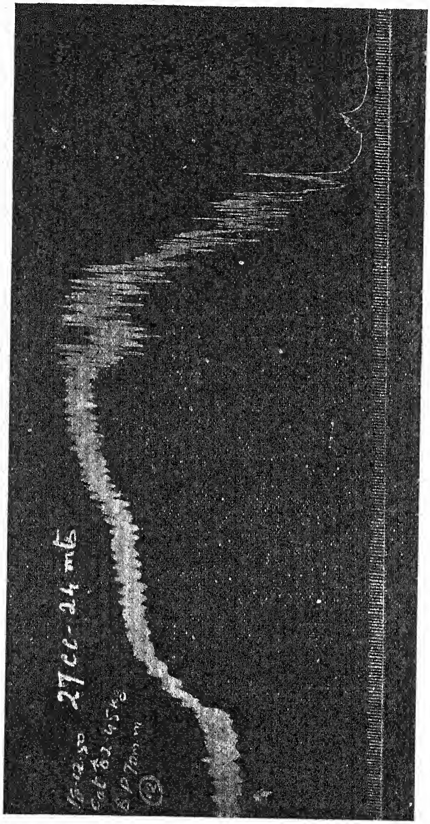
On respiration, the tincture decreases the rate but increses the depth (Plate XX).

Fixation of the Dose. After repeated trials with varying doses of the tincture, it has been assessed that 0·3 c.c. per Kg. body-weight is a safe therapeutic oral dose for canines. 0·6 c.c. per Kg. body-weight is slightly toxic. Retching and emesis should be taken as the first sign of toxicity in the therapeutic administration of the drug. On an average 2 to 12 minims has been fixed as the dose for canines depending on the size and this repeated twice daily for about 20 days did not produce any toxic symptoms.

Discussion

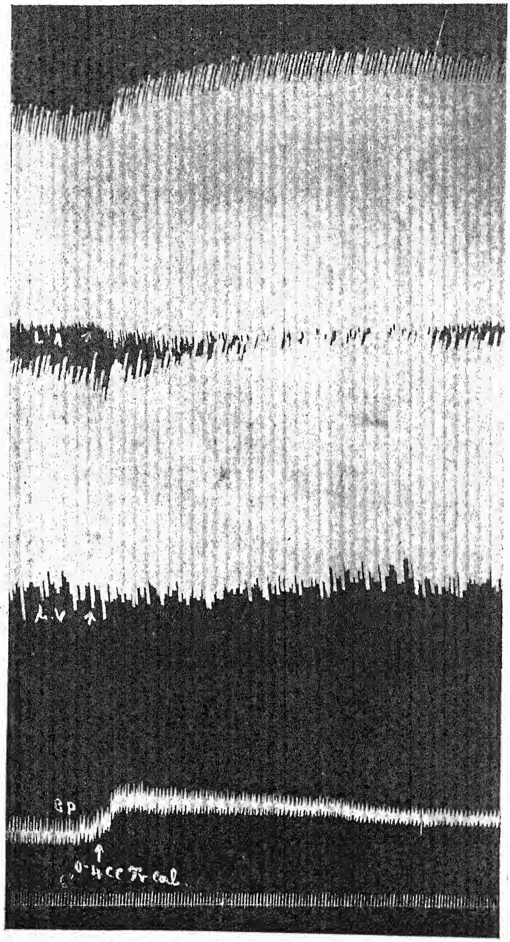
The introduction of standards in the form of dry stable preparation makes comparative methods possible, for every estimation of an unknown sample becomes a comparison with a standard to see how much stronger or how much weaker it is. Since the mixture of the leaf powder was made from different samples, its activity is presumed to be equal to the mean activity of the sample of calotropis. It is possible to establish an international standard from samples obtained from various parts of the world and this may be taken up soon after the therapeutical usefulness has been well established by clinical trials.

There is no evidence that cats vary in sensitiveness to the drug at different times of the year and it is therefore unnecessary to make simultaneous tests of the unknown and the standard. A determination of the average lethal dose per Kg. cat

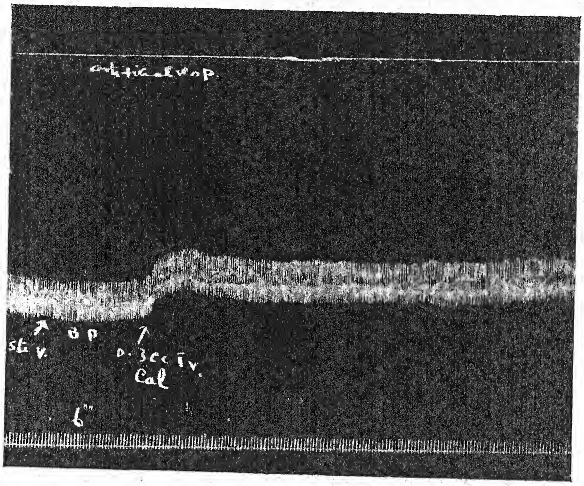


Biological assay of *Tr. calotropis* on a cat.

A record of the carotid blood pressure of a cat. 2·5 Kg. under chloralose anaesthesia. Respiration artificial. *Tr. Calotropis* 1 in 20 was injected through the femoral vein at the rate of about 1 c.c. per minute till the heart was completely arrested. Time marking.—6 seconds.

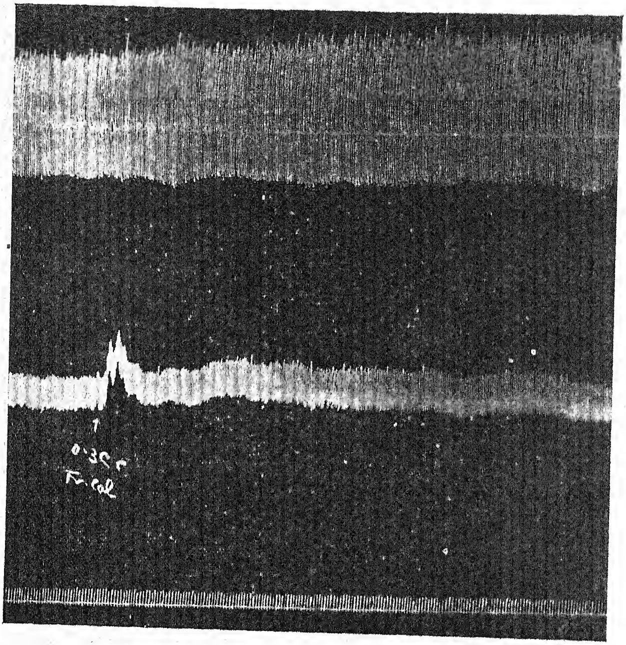


A record of the carotid blood pressure and myocardiogram of a dog, 4 Kg. under paraldehyde anaesthesia, after atropinisation. Note the persistent rise in blood pressure when 0·4 c.c. of *Tr. Calotropis* was given intravenously. The systolic levels of the heart beats are increased whereas the diastole is normal. Up-strokes are systole. L. A.—Left auricle. L. V.—Left ventricle. Time.—6 seconds.



A record of the carotid blood pressure of a dog 5 Kg. under paraldehyde anaesthesia after severing the vagi and atropinisation. Note the rise of blood pressure when 0·4 c. c. of *Tr. Calotropis* was given intravenously.

Time.—6 seconds.



A record of the carotid blood pressure and respiration of a dog 4 Kg. under paraldehyde anaesthesia. Note the rise in blood pressure when 0·3 c. c. of *Tr. Calotropis* was administered intravenously. Also note the increase in the depth and slowing in the rate of the respiration. Upper tracing.—Respiration. Lower.—Blood pressure.

Time.—6 seconds.

of the standard leaf can be made at one time and determination of unknown samples of the leaves can be made at later dates. The results of these determinations are compared with the results previously obtained for the standard.

The dose fixed is in respect of the tincture prepared from the standard powder according to the B.P. method and as it has similar actions as digitalis it will be a cheap but good substitute for digiltalis. Attempts are being made to establish the therapeutic usefulness of the drug by clinical trials.

Summary

The first standard for Calotropis has been prepared and is kept in this laboratory and is available to be compared with unknown samples.

The unit of the standard has been defined as the activity contained in 0·1 G. of the standard powder.

A suitable method of comparison by biological assay has been indicated.

The actions of the tincture has been found to be similar to those of gigantin, the active principle of madar juice.

The dose of the tincture has been fixed for canines.

Acknowledgments

The author's thanks are due to the Indian Council of Agricultural Research, under whose auspices this research was undertaken and to the Director of Animal Husbandry, Madras and the Dean, Government General Hospital and Madras Medical College, Madras, for their keen interests evinced in this work. They are also deeply indebted to Sri. K. Lakshman Rao, B.Sc. (Pharmacy), Research Assistant, Indigenous Drugs Research Scheme, for his valuable technical help throughout.

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ABSTRACTS

MILLER, G. D., FRYE, J. B., Jr., BUSH, B. J., Jr., HENDERSON, P. J. and RUSOFF, L. L. **Effect of Sprinkling on Dairy Cattle** *J. anim. Sci.* (1951) **10**, 4, 961-968

TWO comparable groups of 16 lactating cows (8 Holsteins and 8 Jerseys each were used in a double-reversal experiment to study the effect of cooling by sprinkling on respiration rates, body temperatures and grazing performances. Although both breeds used the shower more as the temperature increased, the Holsteins remained under the shower an average of about 9 per cent more than Jerseys. The average amount of time spent under the shower for both breeds was 2 and 3 times as great at a temperature range of 85° to 90°F. and 92° to 97°F. respectively, as at a temperature range of 80° to 85°F.

Respiration rates were slightly higher for Jerseys than Holsteins, while the reverse was noted for body temperature. The respiration rate of Holsteins and Jerseys having access to shower were 18 and 13 per cent. lower, respectively than for those with only shade. However, the respiration rates of the two groups were very nearly the same after a walk of one-fourth of a mile to the byres.

Sprinkling was effective in producing a lower body temperature for both breeds. Holsteins and Jerseys being sprinkled had body temperatures of 0·7°F. and 0·4°F. lower respectively than did those with only shade. There was an increase of 0·5°F. for each of the breeds, after a walk of one mile to the byres, irrespective of the treatment.

With the temperature range of 80°-85°F., both breeds spent 63 per cent of their time grazing and the use of the shower appeared to have no significant effect on grazing rate. But the grazing rate decreased as the temperature increased and at a temperature range of 92°F. to 97°F. the Holsteins and Jerseys grazed 69 and 68 per cent less respectively, than they did at a range of 80° to 85°F.

Although sprinkling was effective in cooling the cows as borne by the decreasing respiration rates and body temperature and increasing grazing rates, there appeared to be no relationship between the amount of milk produced and treatment. (D.N.)

P. MAHADEVAN **Effect of Environment and Heredity on Lactation** (1951), *J. agric. Sci.* Vol. **41**, 80

VARIOUS environmental factors render it difficult to evaluate the potential breeding worth of animals. In the case of dairy cattle this involves the statistical analysis of production figures of cows in relation to known environmental factors and the subsequent standardisation of these production figures with a view

to eliminating as far as possible the effects of the environmental influences. The records of twelve pedigree herds of Ayrshire cattle numbering about 5,000 were standardized in this way and employed to obtain the estimates of heritability and to study the rate of genetic gain due to selection. The unit of measurement of production employed was the yield during the first 180 days of the lactation period, thus eliminating the effect of the variation in length of current calving interval on milk yield.

The effect of the month of calving on milk yield varied significantly between herds, and it was shown that correction factors for month of calving should be calculated on a within herd basis. The average difference in 180 day yield between summer and winter calvers of all herds was about 10 per cent in favour of winter calvers.

The milk yield of the cow was found to be influenced both by the number of her previous lactations and also by her age at calving. It is found that precentage of correction is the most satisfactory among the types of corrections employed by previous investigators for age.

There was a positive correlation between milk yield and length of preceding calving interval. From an economic point of view, the optimum length of calving interval was found to be 400 days for the first lactation and one year for the subsequent ones. Corrections for the preceding calving interval like those for age were most satisfactory when they were proportionate and not additive.

No significant differences were found among the first three records of a cow in their ability to indicate her actual production capacity. The probable performance of a cow in any lactation was predicted as accurately from the lactation immediately preceding it as from the average of a number of previous lactations.

The average repeatability of milk yield was 0·46 and heritability was of the order 0·25 to 0·30. The probable effect on herd improvement of selecting breeding females was found to be very small, extremely little genetic progress being attained by this method in the twelve herds. (T.M.P.)

RAYMOND N., DOETSCH AND WILLIAM M. SCOTT, (1951). **Bacteriological Aspect of market milk as Consumed in the House hold.** *Milk Plant Monthly*, **40**, 21

THE authors have conducted a bacteriological analysis of market milk received and stored in the house-hold with a view to secure data on the quality of milk as it is consumed in the average homes. A total number of 140 samples collected from different homes in certain parts of New York City, U. S. A., covering different economic levels of the community have been examined for standard plate count, coliform count and for the incidence of the Genus Proteus. Out of 140 samples analyzed, only 20 samples (14 per cent) showed over 30,000 plate count per ml. and only 9 (6 per cent) had 10 or more coliform bacteria per ml. Though the samples from unopened quarts contained some coliform bacteria, those taken from the bottles

previously opened showed high coliform count. Only 4 out of 9 samples from pitchers or glass tumblers showed the presence of coliform. Presence of the Genus Proteus was not noticed in any of the samples. It was further observed that the nature of the container, either of glass or of card board, and the atmospheric temperature had no significant influence on the plate count. The authors conclude that the samples as a whole were surprisingly good from a bacteriological point of view; they ascribe this to the general educational level of the community with regard to concepts of sanitation and cleanliness in the handling of milk and to the extensive use of electric refrigerators for storing the milk. (V. S.)

REVIEWS

THE FEEDING OF FARM ANIMALS IN INDIA

By P. E. Lander, M.A., F.R.I.C., D.Sc., I.A.S.

(*Issued by the Indian Council of Agricultural Research—Published by Macmillan & Co., Ltd., Calcutta, India, 1949, pp. 543, Price Rs. 14.*)

THIS is a first of the series of Animal Husbandry Manuals issued by the Indian Council of Agricultural Research. Following on the meeting of the Crops and Soils Wing of the Board of Agriculture and Animal Husbandry in India (1942) the preparation of this manual was undertaken by Dr. P. E. Lander a d although this volume was ready for publication in 1943, but due to unfavourable conditions created by World War II and division of the country, this could not be published earlier than 1949. The Manual is a very welcome addition to the scanty literature on indigenous cattle feeds and feeding in this country. The manual is divided up in 16 chapters and covers 492 pages with 45 photographs, diagrams and charts besides 5 appendices. The manual gives useful information on all problems connected with the feeding of farm animals and is indispensable for scientists working on the subject, students studying the subject and practical cattle breeders.

The first two chapters are devoted to the types of soil and composition of various feeding stuffs. It has been shown how the weak soil produces food and fodder of a deficient nature or of less nutritive quality and its ultimate unhealthy effects on the human and animal life. Methods are indicated how to effect improvement of soils to obtain healthy and better quality crops and raise yields. Attention to this aspect alone if given effect to by cultivators will indirectly improve the economic condition of the country.

General principles of maintenance of animals and poduction from them and their feeding standards and balanced rations are next dealt with in detail. The feedings standards enable one to express the nutritive value of foods and the food requirements of animals in some common terms. Rations may be changed and the proportions of their relative constituents may vary, hence for the compilation of rations with definite standards which can be expressed in terms of energy of nutrients for various purposes are indicated. This enables one to effecting maximum economy in the feeding of animals and at the same time to maintain health and productivity.

A detailed account of the feeding stuffs, their preparation, storage and an account of harmful feeds and famine fodders is given in the next three chapters. In discussing the preparation and storage a comprehensive account of hay and silage making, its storage and time at which they should be fed to the animals, is given. The qualities and varieties of grass are also given which merit best for hay and silage making. Mention is made of plants which are poisonous in varying degree when fed

to animals. A list of hill and plains fodder trees and plants with their merits and demerits as famine fodders are indicated and for this account alone the manual is valuable and indispensable.

The cattle being generally poor milkers in this country are uneconomical to maintain. The fact however remains that the majority of cattle do not get either adequate food or of the right type. The manual gives a detail and general plan for the feeding of milch and draught animals and other livestock including horses, mules, sheep, goat, pigs, camels and poultry.

The computing of rations for different class and types of animals on most economical basis are then dealt with, all of which if followed carefully will greatly help in the rural uplift and improve the economic conditions.

The manual is a valuable reference book written systematically in simple English. Dr. Lander, the author, has many years, experience of, and research in, animal nutrition in India. He was Agricultural Chemist in the Government of the United Punjab and later Principal of Lyallpur Agricultural College, and as such the publication is an authoritative statement of the present position of livestock nutrition in the country and meets the need for a handy, informative and reliable book on the subject. The manual is well printed and is recommended to those who require an authoritative book on the feeding of farm animals including poultry. (M.R.M.)

NOTES FOR INTRODUCTORY COURSES IN GENETICS

By Charlotte Auerbach, Ph.D., D.Sc.

Published by Oliver and Boyd Edinburgh and London (1951, 3rd ed., pp. 42, Price 2s.)

HERE is a concise and easy-to-understand booklet dealing with the basic concepts and laws of genetics. These ' Notes ' are the outcome of a successful teacher's experience extending over a long period, of the needs of the elementary students attending short courses on the subject. The object of this booklet is, as has been explained by the author herself; in the foreward, is not to replace any text book on the subject or to serve as a popular introduction to the science but is intended to supplant the notes, usually taken by the students themselves so that they can adequately devote their attention to the discussions. This object has been well fulfilled. The science of Genetics is full of teminologies which require great accuracy and clarity in understanding. As such, this booklet should be most welcome to the students as it will relieve them from tedious note-takings. The author is to be complimented for achieving both brevity and clarity in dealing with basic concepts and laws of genetics. Technical terms have been very well defined. Many important topics have been explained very lucidly in a concise form. Meiosis, though without details, is well explained in para. 8, the Mendel's laws in paras. 11 and 23, blood groups in para. 18 and sex determination in para. 19. The booklet is full of illustrative examples. The elementary Agriculture, Animal Husbandry, Veterinary and Medical students will find the two short appendices at the end, very useful. (P.B.)

SUCCESSFUL POULTRY MANAGEMENT

By Morley A. Jull.

(Published by McGraw Hill Rural Activities Series, 437 pp. 500)

THE author is a national authority in poultry work in the U. S. A. He is well known as a teacher and poultry author to the students of Animal Husbandry and Agriculture all over the Globe.

The book should be extremely useful to the graduate students, farmers, commercial poultry producers, hatchery men, feed and equipment manufacturers, and as a matter of fact to every poultry minded man.

The title of this book is most appropriate as in all the pages is emphasized the fact that success in poultry farming is mainly a matter of efficient management. The book presents to poultry keepers up-to-date information on husbandry practices and marketing methods affecting returns from the poultry business, large or small. Production of eggs and table fowls economically and how to market these highly perishable commodities to the fullest advantage are discussed in detail and in a popular manner. The author stresses on factors affecting the quality of eggs and poultry meat because the producers hardly appreciate their role in determining prices paid for poultry products in the course of marketing. Illustrations are most apt, carefully selected and represent an outstanding feature of the book.

The various facts and working details are drawn from research and the wealth of knowledge gained by poultry raisers. What kind of stock to keep, is treated in a unique and different manner. New score cards are included for judging hens on the basis of laying potentialities and for breeding stock for standard bred and utility characters. Practical suggestions are also enumerated for broiler as well as egg production. Special emphasis is placed on the nutrition of chickens as feed costs represent about 60 per cent. of the total cost of production. Culling birds of all classes to maintain the efficiency of stock at a high level is discussed under different headings in a manner which laymen can easily follow.

The Indian poultry keeper is badly in need of an up-to-date text book on poultry keeping and this book of Dr. Jull should serve the purpose well. (S.G.I.)

PRINCIPLE OF MILK PRODUCTION

By William Barbour Nevens.

(Published by the McGraw Hill Book Co., Inc., New York, 1951, pp. 443, Price 5·00)

THIS is the first edition of an introductory book on the subject. Through this compact volume of 443 pages the author has directed his efforts to cater the basic knowledge required for collegiate students on the principles of milk production, as also to provide the dairy farmer a reference book to serve as a guide in the establishment and day to day management of a profitable milk-production enterprise.

The text is comprised of 7 parts and 26 chapters. Part I begins with a survey of the importance of dairy in Agriculture. Part II contains 11 chapters dealing with milk production records, calculation of balanced rations and feeding of dairy

cattle, general consideration in selection, systems of breeding, artificial insemination and management of dairy cattle. Part III contains a description of important dairy-cattle breeds and selection of individual animals on the basis of physical characteristics. Part IV contains 4 chapters devoted to a systematic treatment of such important aspects as the structure and development of cow's udder, mechanism of milk secretion, constituents of milk and factors influencing them and clean milk production. Part V having 2 chapters deals with the marketing of dairy products from the farm, the price and price plans and the economics of milk production. Part VI contains 5 chapters on the soil management, pasture systems, production and conservation of fodder crops. Lastly, a chapter is devoted to the designing of bars and milk houses.

Prof. Neven's book is written in simple and good style and the subject matter has been interpreted with great latitude. Special topics such as the simple and practical method of calculating balanced rations, good soil management and grassland farming, not commonly presented in a book of this type, enhances the value of this publication. It is a useful book for the collegiate student, teacher and all those interested in dairying. The printing and get-up of the book speak of the obvious thoroughness of the American publishers and printers. (K.C.S.)

FARMER AND STOCK BREEDER YEAR BOOK 1952

(Published by Farmer and Stock Breeder Publications Limited, 76th edition (1951) Dorset House, Stamford Street, London, S.E.I. Price 10s. 6d., net postage 6d. pp. 412, over 170 illustrations)

MORE useful information than ever before is included for stock breeders and those interested in any phase of livestock and general farming. The table of contents includes a valuable breeders' table—special article of topical interest to farmers and breeders of livestock—pedigree year in pictures, a section on poultry, and a unique reference section with its own index.

The breeders' table is included for the first time and is a valuable calendar for all the 12 months to record service date and an easy calculation and facility for recording the birth date in respect of mare, cow, ewe and sow.

The special article section is well illustrated and is informative and each is written by a contributor possessing considerable experience and knowledge in their respective fields of work. The articles cover such wide range of subjects as, seedtime to harvest, sheep, pigs, controlled grazing, silage making, root crop, feeding dairy cows, horticulture problems, and farming equipment.

The pedigree year in pictures is a section of numerous very beautifully got up photographs of the year's best and topmost champions which parade out of the year's best livestock shows. The stock of fine British cattle, pigs, sheep and horses of which Britain may take pride can be well appreciated and studied in this section.

The poultry section is devoted to producing turkeys all the year round and the policy of selling out pullets in spring. (M.R.M.)

The reference section is perhaps the most valuable of all as it gives full information to all interested as to who is who in official and semi-official organisations and institutions, principal shows, Artificial Insemination centres, Insurance, weather, rations, composition of feeding stuffs, British farming records, statistics, prices, pedigree sales and buyer's guide, etc. If for no other reason, the book is worth more than its value or all the information in one place that anyone interested in livestock may obtain and be proud to possess.

The book is well printed and bound. Fine quality paper is used throughout. A galaxy of all kinds of advertisements on farming are included. The book is of value and interest to all concerned with any phase of livestock industry. (M.R.M.)

NOTE.

XV—INTERNATIONAL VETERINARY CONGRESS, 1953.

THE members of the veterinary profession in India will be interested to know that the Organising Committee of the International Veterinary Congress has announced that the XV International Veterinary Congress will be held in Stockholm from the 9th to the 15th August 1953. Stockholm, the Capital city of Sweden is to be the venue of this Congress. The Organising Committee of the XV International Congress has drawn up a preliminary programme according to which the scientific part of the Congress will comprise of a Plenary and Sectional meetings. The Plenary meetings, will cover a wide range of subjects of Veterinary Science like Infectious Diseases, Mastitis in cattle, Metabolic Disturbances, Food hygiene and Comparative pathology.

To facilitate detailed scientific discussions it is proposed to split up the Sectional meetings into 9 separate Sections which will cover all the important aspects of Veterinary science and Animal Husbandry such as Infections and parasitic diseases, Poisonings, Problems of Metabolic disturbances, Deficiency diseases, Allergies, Diagnosis, Therapy and Surgery. The Physiology and Pathology of reproduction and Lactation; Animal Husbandry subjects comprising of breeding including artificial insemination, feeding, and management will form separate sections. One of the most important sections will be on the co-ordination of Internatinal Veterinary Problems. Under this the Congress will discuss International standardization of sero-diagnostic methods of bacteriological preparation like sera, vaccines and allergins; transport of quarantine regulations, international transmission of semen; and the International co-operation for producing veterinary educational films.

The above preliminary programme is subject to ratification by the Permanent Commission for International Veterinary Congress. Thereafter the Organising Committee will issue invitations to read papers giving particulars concerning the names of plenary lecturers and the titles of the papers. Invitation to the membership of the Congress will be distributed in August 1952.

The address of the Organising Committee is: XV International Veterinary Congress, Organising Committee, c/o Isaksson, State Veterinary Medical Institute, Stockholm 50, Sweden.

MGIPC—M—25 AR—11-3-53—830.

ORIGINAL ARTICLES

THE NUTRITIVE VALUE OF THE INDIGENOUS GRASSES OF ASSAM

VI. THE GRASS *JOY-JOHA* (*ISCHAEMUM RUGOSUM* SALISB) AS A CATTLE FEED

By B. K. DAS AND N. C. MUKHERJEE, Animal Nutrition Research Scheme, Gauhati

(Received for publication on 27 December 1951)

IN a series of papers, recently Talapatra [1949 and 1950] showed that although the indigenous grasses are deficient in some of the indispensable mineral elements like calcium and phosphorus yet they could profitably be introduced in the dietary of cattle in various forms; in the case of the aquatic grasses, however, he and his associate [Talapatra *et al*, 1949] observed that green feeding led to considerable worm troubles, while the mature hay from these grasses could be fed to any type of cattle without the least fear of helminthic infection.

Most of the investigations so far reported from this laboratory seem to indicate that the animals thriving on the indigenous grasses suffer from a sort of chronic calcium deficiency, because of the very low content of this mineral in the herbage.

Recently we have come across a kind of semi-aquatic grass which appears to be fairly rich in both calcium and phosphorus even at the flowering stage. This is commonly known as *Joy-Joha* (*Ischaemum rugosum* Salisb). It is an annual grass, tall, erect or ascending in robust tufts and branching at the base. The branches are often 2-nate, compressed, very glabrous and swollen at the top. Leaf-blades are soft, flat, linear or almost lanceolate. The culms are 6 to 39 inches tall. It looks more or less like 'Bao' (spring) paddy. It is available from June to November and flowers during the middle of October, when the other grasses in this part turn woody and fibrous. As the weather is generally favourable for hay making in October, it can be conserved by the 'Tripod System' in large quantities.

An attempt has been made in the present paper to study the chemical composition and the nutritive value of this grass when fed both as a green fodder and a hay at the flowering stage.

(a) *As a green fodder* (*flowering stage*)

Four adult Assamese bullocks weighing about 570 lbs. were taken and fed exclusively with the grass for a period of 31 days; and as usual urine and faeces were collected during the last 10 days. Residues left during the collection period were kept separately for each animal and accounted for.

The chemical composition, the dry matter consumption, the digestibility coefficients and the calcium, the phosphorus and the nitrogen balances have been shown in the following tables.

TABLE I

The chemical composition of Joy-Joha (on dry basis)

Nutrients	Whole plant	Stem (residue)
Crude protein	7·13	4·03
Ether Extract	2·50	1·27
Ash	10·07	7·87
Crude fibre	29·80	35·18
Nitrogen free extract	50·50	51·6
Total carbohydrates	80·30	86·78
Organic matter	89·93	92·13
Calcium (Ca)	0·38	0·24
Phosphorus (P)	0·31	0·18

The chemical composition of the grass even at the flowering stage indicates a fairly high proportion of protein while the content of calcium and phosphorus having an ideal Ca/P ratio is much higher than those previously reported from this laboratory.

TABLE II

Dry matter consumption of green Joy-Joha

Animal number	Body weight (lb)	Dry matter consumed (lb)	Dry matter consumed per 100 lbs of body-weight (lb)	Average (lb)
1	565	11·05	1·96	
3	570	10·60	1·86	
4	564	10·92	1·94	1·89
5	557	10·38	1·81	

The average dry matter consumption of 1·89 lbs per 100 lbs of body weight indicates that the grass is highly palatable as a green fodder even at the flowering stage.

Table III

Digestibility coefficients of the organic nutrients

Animal number	Amount supplied in feed	Amount left in residue	Intake	Excreted in faeces	Amount digested	Digestibility coefficients	Average digestibility coefficients
			Dry Matter				
	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)	per cent	
1	7067	2057	5010	2173	2837	57	
3	7067	2258	4809	1901	2908	61	
4	7067	2115	4952	2061	2891	58	58
5	7067	2355	4712	2044	2668	57	
			Crude protein				
1	503·9	82·9	421·0	161·0	259·9	62	
3	503·9	91·0	412·9	147·8	265·1	64	
4	503·9	85·2	418·7	171·4	247·3	59	62
5	503·9	94·9	409·0	160·4	248·6	61	
			Ether extract				
1	176·7	26·1	150·6	54·8	95·8	64	
3	176·7	28·7	148·0	41·8	106·2	72	
4	176·7	26·9	149·8	41·2	108·6	73	71
5	176·7	29·9	146·8	39·5	107·3	73	
			Crude fibre				
1	2106·0	724·1	1381·9	528·0	853·9	62	
3	2106·0	794·8	1311·2	452·4	858·8	66	
4	2106·0	744·5	1361·5	500·8	860·7	63	63
5	2106·0	829·0	1277·0	504·9	772·1	60	
			N-free extract				
1	3568·8	1061·4	2507·4	1080·0	1427·4	57	
3	3568·8	1165·1	2402·7	944·8	1458·9	61	
4	3568·8	1091·3	2477·5	995·5	1482·0	60	59
5	3568·8	1215·1	2353·7	983·2	1370·5	58	
			Total carbohydrates				
1	5674·8	1785·5	3889·3	1608·0	2281·3	59	
3	5674·8	1959·9	3714·9	1397·2	2317·7	62	
4	5674·8	1835·8	3839·0	1496·3	2342·7	61	60
5	5674·8	2044·1	3630·7	1488·0	2142·7	59	

The data in Table III show that the protein digestibility from the green *Joy-Joha* even at the flowering stage is quite satisfactory.

The digestibility of ether extract however is very high and is not generally to be experienced with the indigenous grasses.

The digestibility of crude fibre appears to be normal. As regards N free extract andtotal carbohydrates all the animals behaved uniformly.

From the average digestibility coefficients, the starch equivalent has been calculated according to the method of Kellner and is shown below along with some of the cultivated fodder plants. The average dry matter is 28·27 lbs per 100 lbs of the green feed fed to the animals.

TABLE IV

Digestible nutrients in Joy-Joha (*flowering*) *as compared to cultivated fodder plants*

—	Digestible nutrients per 100 lbs of the green stuff	
	D.P.	S.E.
Joy-Joha (flowering)	1·25	10·88
Guinea (young)	1·11	7·30
Napier	0·96	9·6
Jowar	0·84	7·2
Guara	1·33	6·2

It is quite evident, therefore, from the data shown above that the grass *Joy-Joha* is in no way inferior to any one of the cultivated fodder plants.

Calcium, phosphorus and nitrogen balances under Joy-Joha *feeding.*—The calcium, phosphorus and nitrogen balances have been shown in Table V.

TABLE V

Calcium, phosphorus and nitrogen balances of the animals

Animal number	Total intake	Excreted in faeces	Excreted in urine	Total excreted	Balance
			Calcium		
	(gm.)	(gm.)	(gm.)	(gm.)	(gm.)
1	21·91	17·28	2·63	19·31	+2·60
3	21·43	14·36	0·75	15·11	+6·32
4	21·78	17·25	1·67	18·92	+2·86
5	21·20	17·77	1·25	19·02	+2·18
			Phosphorus		
1	18·21	10·16	0·12	10·28	+7·93
3	17·85	9·57	0·14	9·71	+8·14
4	18·10	9·61	0·15	9·76	+8·34
5	17·67	9·99	0·10	10·09	+7·58
			Nitrogen		
1	67·35	25·78	14·75	40·53	+26·82
3	66·06	23·65	14·39	38·04	+28·02
4	66·98	27·43	14·25	41·68	+25·30
5	65·43	25·67	14·82	40·49	+24·94

It may be seen from the above data that definite positive balances of calcium and phosphorus are assured under *Joy-Joha* feeding. The heavy retention of nitrogen is perhaps purely of a temporary nature and is generally observed with adult animals kept on a high plane of nutrition. The positive calcium and phosphorus balances observed in this case are not generally found with most of the indigenous grasses in this part of the country particularly when fed as a single feed, [Talpatra, 1949, 1950]. It may possibly be due to a high intake of protein. A liberal intake of protein and the nature of protein seem to have some influence on the absorption of both calcium and phosphorus [Lehmann *et al* 1942, Mc Cance *et al* 1942, Desikachar, H. S. R. and Subrahmanyan. V. 1949]. Better assimilation of minerals is sometimes attributed to improved crude fibre digestibility [Maynard, 1937]. In this case the crude fibre digestibility also may probably have some effect on the positive retention of calcium and phosphorus.

(b) Joy-Joha *as hay* (*flowering stage*)

The grass was turned into hay by the well-known 'Tripod System'. The hay retained good green colour and no sign of decomposition was noticed.

Three adult Assamese bullocks weighing about 525 lbs were taken and fed exclusively with the hay for a sufficiently long period which was then followed as usual by a ten day period of collection of both faeces and urine. Residues left during the collection period were kept separately and accounted for. The chemical composition, the dry matter consumption, the digestibility coefficients and the calcium, the phosphorus and the nitrogen balances have been shown in the following tables.

TABLE VI

Chemical composition of Joy-Joha *hay* (*on dry basis*)

——	Whole plant (hay)	Stem (residue)
Crude protein	6·63	3·39
Ether extract	2·18	1·09
Ash	9·63	6·71
Crude fibre	33·72	42·95
N-free extract	47·84	44·96
Total carbohydrates	81·56	87·91
Organic matter	90·37	93·29
Calcium(Ca)	0·37	0·245
Phosphorus(P)	0·24	0·164

The chemical composition of the hay is almost identical with that of the green grass.

TABLE VII

Dry matter consumption of Joy-Joha *hay*

Animal number	Body weight	Dry matter consumed	Dry matter consumed per 100 lbs of body-weight	Average
	(lb)	(lb)	(lb)	(lb)
1	525	7·5	1·4	
3	532	7·5	1·4	
4	523	7·7	1·5	1·4

Contrary to expectation, the same grass when turned into hay almost at the same stage of growth was not much relished, as indicated by the decreased consumption of dry matter. It may be seen that the dry matter consumption was decreased by about 25 per cent.

TABLE VIII

Digestibility coefficients of the organic nutrients of Joy-Joha *hay*

Animal number	Dry matter	Crude protein	Ether extract	Crude fibre	N-free extract	Total carbohydrates
1	50	44	56	65	47	54
3	56	46	60	68	54	60
4	49	36	50	65	47	54
Average	52	42	55	66	49	56

A perusal of the data in Table VIII shows that the digestibility figures for a mature grass-hay are quite satisfactory. From the average digestibility coefficients the net energy value of Kellner has been calculated and shown along with some of the well-known hays of the country.

Names of hays	Digestible nutrients per 100 lb. of the raw stuff	
	D.P. (lb)	S.E. (lb)
Joy-Joha hay	2·4	26·7
Arali hay	2·0	23·9
Anjan hay	1·5	29·2
Bolaram hay	2·0	27·8
Jowar hay	2·5	24·8
Guinea grass hay	1·9	19·9
Usar hay	2·31	17·5
Ambala hay	2·2	23·5

It is evident from the above figures that the *Joy-Joha* hay is comparable to most of the well-known hays of the country.

Calcium, phosphorus and nitrogen balances under Joy-Joha *hay feeding.*—Under the same dietetic conditions of the above digestibility trial calcium, phosphorus and nitrogen balances were determined and shown below in the Table IX.

TABLE IX

Calcium, phosphorus and nritogen balances per day

Animal number	Intake (gm.)	Excreted in faeces (gm.)	Excreted in urine (gm.)	Total excreted (gm.)	Balance (gm.)
			Calcium		
1	13·74	14·34	1·77	16·11	—2·37
3	13·70	12·51	1·73	14·24	—0·54
4	13·94	14·71	1·89	16·60	—2·66
			Phosphorus		
1	8·69	8·49	0·08	8·57	+0·12
3	8·69	7·08	0·10	7·18	+1·51
4	8·82	8·18	0·13	8·31	+0·51
			Nitrogen		
1	40·81	22·98	10·05	33·03	+7·78
3	40·71	22·02	10·04	32·06	+8·65
4	41·24	26·26	9·11	35·37	+5·87

The low dry matter consumption is perhaps responsible for the negative calcium balance. The nitrogen balance is definitely positive which indicates that the hay is a maintenance ration. In these cases the digestible protein provided was at the rate of 0·46 lbs per 100 lbs body weight. Though the balance of calcium is negative, the phosphorus balances were all positive showing possibly that much of the phosphorus present in the grass is in organic combination and is closely related with the protein metabolism.

SUMMARY

The grass *Joy-Joha* can profitably and successfully be utilized in the dietary of cattle till November as a green feed. At the flowering stage it contains 7·13 per cent of crude protein on dry basis. The digestibility coefficients of crude protein, ether extract and total carbohydrates are 62, 71 and 60 respectively. The digestible protein and the starch equivalent (Kellner) per 100 lbs of the green stuff are found to be 1·25 and 10·88 lbs respectively. Unlike most of the indigenous grasses in this

part of the country, positive balance of both calcium and phosphorus were assured when the grass was fed as a green fodder.

Joy-Joha hay at the flowering stage was not much relished by cattle. The hay prepared from the grass at the flowering stage contains 6·63 per cent of crude protein. The digestibility coefficients of crude protein, ether extract and total carbohydrates are 42, 55 and 56 respectively. The digestible protein and the starch equivalent (Kellner) per 100 lbs of the stuff are 2·4 and 26·7 lbs respectively.

A positive retention of calcium is not assured but the hay provides a maintenance ration with regard to both protein and phosphorus.

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THE UTILISATION OF MANGO-SEED KERNEL AND *JAMAN* SEED MEAL IN A SIMPLIFIED POULTRY RATION FOR GROWING CHICKEN

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In view of the acute food shortage in the country, investigations into the proper utilisation of the waste materials in the poultry ration are of great importance for the poultry industry in India. In a previous investigation Bose, Thakral and Narayanan [1950] concluded that mango-seed kernel, which is generally thrown away as a waste material, could be utilised by replacing as much as 20 per cent of yellow maize meal in the laying ration with beneficial results. The inclusion of *Jaman* seed meal, on the other hand, had great depressing effect on the egg production. The present investigation has been undertaken to study the effects of the addition of mango-seed-kernel and *jaman* seed meal to a simplified poultry ration for growing chickens.

Experimental

A total of 45 Rhode Island Red chicks hatched in May, 1950, were divided into three comparable groups and fed on the experimental diets for a period of eight weeks. The birds were fed on all-mash rations in a wet crumbly state according to appetite in the morning, noon and afternoon. Broken limestone, succulent green food and fresh clean water were supplied *ad lib.* during the experimental period. Group I was fed on the basal ration consisting of yellow maize meal 55 parts, wheat bran 25 parts, groundnut cake meal 19 parts and common salt 1 part. Group II received all-mash ration in which 20 per cent of yellow maize meal of the basal ration was replaced by mango-seed-kernel. Group III was fed on all-mash ration in which 20 per cent of yellow maize meal of the basal ration was replaced by *jaman* seed meal. Cooked meat offal was mixed with the mash and fed to the birds in all the three groups as protein supplement at the rates of 1 part of meat offal for every 2 parts of the mash. The quantity of meat offal required every day was cooked for about an hour in a small quantity of water and run through a mincing machine prior to mixing with the mash.

The average percentage chemical composition of the experimental ration is shown in Table I.

TABLE I

The average chemical composition of the experimental growing rations on dry basis

Feed	Crude protein	Ether Extract	Ash	Carbo-hydrates
Mash—				
Group I	18·3	4·1	3·7	73·9
Group II	17·9	5·7	4·4	72·0
Group III	17·0	3·6	5·1	74·3
Meat Offal	64·5	30·7	4·8	..

The chickens were weighed individually at weekly intervals. The average weights of the males and females from 0 to 8 weeks are recorded in Table II.

TABLE II

Average weights in oz

	Group I		Group II		Group III	
	Male	Female	Male	Female	Male	Female
Day old	1·1	1·5	1·4	1·4	1·4	1·[illegible]
1 week	1·5	1·7	1·5	1·6	1·7	1·6
2 weeks	2·4	2·9	2·6	2·4	2·8	2·6
3 weeks	3·8	4·4	4·0	3·8	4·4	3·8
4 weeks	4·8	5·3	5·4	4·9	5·6	5·0
5 weeks	7·1	7·4	7·6	6·7	7·3	7·0
6 weeks	9·9	9·4	9·8	9·6	10·1	8·6
7 weeks	13·0	11·7	12·5	11·6	11·5	10·3
8 weeks	15·1	13·3	14·1	13·7	15·2	13·4

The average food consumption in ounces per bird per day and the corresponding figures of the utilisation of food in ounces, per ounce live weight gain, are recorded in Table III.

TABLE III

Food consumption and utilisation

Weeks	Average food consumption per bird per day (oz)			Food consumption in ounces per ounce live-weight gain		
	Group I	Group II	Group III	Group I	Group II	Group III
1	0·17	0·24	0·21	7·93	11·20	7·35
2	0·27	0·28	0·33	1·80	2·06	2·20
3	0·43	0·40	0·46	2·08	2·00	2·30
4	0·42	0·47	0·51	3·10	2·63	2·98
5	0·71	0·73	0·94	2·26	2·56	3·55
6	0·93	0·98	1·14	2·71	2·05	3·63
7	1·23	1·55	1·43	3·19	3·09	6·46
8	1·60	1·75	2·10	6·05	6·62	4·32

The general health of the birds in all the three groups, as judged by handling and appearance, was satisfactory. The mortality percentage during the experimental period for groups, I, II and III were 6·6, 0, 13·2 respectively.

The statistical examination of the data of Tables II and III are shown in Table IV.

TABLE IV

Statistical analysis

(*Analysis of variance*)

Sample	Magnitude of variation	Remarks
Table II—		
(*a*) Body weights of male birds	F=0·003	As the points at the 5 per cent and 1 per cent for the distribution of F are 3·47 and 5·78 respectively, the groups or sub-samples evidently come from the same population. The differences are, therefore, not significant.
(*b*) Body weights of female birds	F=0·025	Do.
Table III—		
(*a*) Food consumption	F=0·18	Do.
(*b*) Food consumption per oz live weight gain.	F=0·14	Do.

Discussion

At eight weeks the average weights in ounces of the males in Groups I, II and III were 15·1, 14·1 and 15·2 respectively. The corresponding figures for females were 13·3, 13·7 and 13·4. Although the experiment was carried out in the monsoon season, which is not very suitable for growing chickens in the Northern India, it is remarkable that the rate of growth of the chickens compared very favourably with the rate of growth usually obtained during the winter months. At no stages of growth there was any significant difference between the average weights of Group I, II and III. Both mango-seed-kernel and *Jaman* seed meal can, therefore, replace 20 per cent of the yellow maize meal in growing ration without any detrimental effect. This is rather interesting because similar experiments carried out on laying hens [Bose, Thakral and Narayanan, *loc. cit.*] revealed that *jaman* seed meal had great depressing effect on egg production. The factor present in *jaman* seed meal, which adversely affected egg production, had therefore no detrimental effect on the growth of chickens.

The average food consumptions were satisfactory and very similar for all the three groups. The utilisation of the experimental rations in ounces per ounce live weight gain did not show any significant difference.

Summary

The inclusion of mango-seed-kernel and *jaman* seed meal by replacing 20 per cent of yellow maize meal separately in a normal growing ration showed no detrimental effect. The rate of growth, food consumption and food utilisation were similar in all the three groups.

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PHYSIOLOGICAL STUDIES ON THE BLOOD OF DOMESTIC ANIMALS III. THE NORMAL BLOOD PICTURE OF THE KUMAONI BULLOCK*

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The importance of the study of the normal blood composition of animals in nutritional and physiological research has been emphasized by Sen and Roy [1933], Kehar [1940] and Kehar *et al.* [1940, 1945, 1951*a*, 1951*b*]. The factors likely to cause variations in blood from the normal range may be of food, breed or climate. Mullick and Pal [1943] observed variations in the blood composition of Hariana and Dhanni breeds. Since extensive nutritional investigations are carried out on Kumaoni bullocks, which are commonly found in the lower Himalayan ranges and are very economical and docile, it was considered desirable to study in detail their normal blood components.

Experimental

Twenty four healthy adult Kumaoni bullocks were selected for analysing the morphological, organic and inorganic constituents of blood. They were maintained on the Institute basal ration consisting of rape cake and wheat bhoosa throughout the experimental period. The observations were recorded during the

Table I

Showing the morphological constituents of Kumaoni bullock as compared to Hariana and Dhanni bullocks

Constituents	Kumaoni bullock			Hariana bullock		Dhanni bullock
	Maximum	Minimum	Mean with standard error	Mean [Kehar and Murty, 1945]	Mean [Mullick and Pal, 1943]	Mean [Mullick and Pal, 1943]
Haemoglobin gms/100 ml. blood	9·2	6·2	7·4±0·18	11·0	10·8	12·5
Red blood corpuscles—millions per c.mm. of blood	8·4	4·4	6·5±0·24	7·7	8·0	7·9
White blood corpuscles—thousands per c.mm. of blood	11·9	6·4	8·4±0·33	7·7	8·4	8·0
Cell volume—per cent	46·2	24·6	35·3±1·34	50·6	..	..
Corpuscular values (calculated)—						
Mean corpuscular volume (M.C.V) cubic microns	63·5	43·4	54·6±1·17	68·7	..	..
Mean corpuscular haemoglobin (M. C. H.) micromicro grammes	14·3	10·1	11·6±0·29	14·0	..	..
Mean corpuscular haemoglobin concentration (M. C. H. C.) per cent	25·3	19·0	21·2±0·40	21·8	..	..

* Read at the Indian Science Congress Session 1948—*Proc. Ind. Sci. Cong.* 35. No. 3, 96.

3

summer months. Blood was drawn from the jugular vein before the morning feed was given. The morphological constituents and blood sugar (total reducing substances) were estimated in the whole blood. Neutral potassium oxalate was used as the anticoagulant and the necessary correction made in calculating the cell volume. The inorganic constituents, *viz.*, calcium, inorganic phosphorus, magnesium, sodium, potassium and chlorides, and proteins were estimated in blood serum.

Results and discussion

The morphological constituents were determined by the methods described by Napier and Das Gupta [1946]. The range and the average with standard error of these constituents are shown and compared with those of the other breeds of cattle in Table I. It is evident from the Table I that the Kumaoni bullocks, as compared to the Hariana and Dhanni bullocks, recorded significantly lower values for all the haematological constituents studied except the white blood corpuscles.

Haemoglobin. The method of Newcomer as described by Hawk, Oser and Summerson [1947] was used in estimating the haemoglobin content of the animals. It varied from 6·2 to 9·2 with an average value of 7·4±0·18 gms. per 100 ml. of blood. This figure is definitely lower than the one reported by Kehar and Murty [1945] and Mullick and Pal [1943] for Hariana and Dhanni bullocks respectively.

Red blood corpuscles. The mean red blood corpuscular count was 6·5±0·24 within a range of 4·4 and 8·4 millions per cubic millimeter of blood. Kehar and Murty [*loc. cit.*] and Mullick and Pal [*loc. cit.*] recorded average values of 7·7 and 8·0 millions per c.mm. of blood respectively for the Hariana bullocks. The latter authors gave a value of 7·9 millions per c.mm. in the blood of the Dhanni bullocks.

White blood corpuscles. The maximum and minimum count for the white blood corpuscles were 11·9 and 6·4 thousands per cubic millimeter of blood with an average value of 8·4±0·33. This figure is in agreement with the value given by Mullick and Pal [*loc. cit.*] for the Hariana bullocks, but is higher than the values reported for the Dhanni bullocks.

Corpuscular volume. The average corpuscular volume was 35·3±1·34 per cent with a range of variations from 24·6 to 46·2.

Corpuscular values (*calculated*). The average mean corpuscular volume is 54·6±1·1 cubic microns with a range of 43·4 to 63·5 cubic microns. The mean corpuscular haemoglobin varies between 10·1 and 14·3 micro micro grammes, with an average of 11·6±0·29. The maximum and minimum corpuscular haemoglobin concentration is 25·3 and 19·0 per cent respectively, with an average of 21·2±0·10. All the corpuscular values are lower than those reported by Kehar and Murty [*loc. cit.*] for the Hariana bullocks.

Organic and inorganic constituents. The range and the average with standard error of the organic and inorganic constituents as compared to those of the two breeds of bullocks are presented in Table II.

Sugar (*total reducing substances*). Blood sugar was determined by the method Hagedorn and Jensen as described by Hawk *et al.*, [*loc. cit.*].

TABLE II

Showing the organic and inorganic constituents of Kumaoni bullock as compared to Hariana and Dhanni bullocks.

Constituents	KUMAUNI BULLOCK		Mean with standard error	HARIANA BULLOCK		DHANNI BULLOCK
	Maximum	Minimum		Mean [Kehar and Murty, 1945]	Mean [Mullick and Pal, 1943]	Mean [Mullick and Pal, 1943]
Organic constituents						
Sugar (Total reducing substances) mg/100 ml. blood.	102·3	70·6	88·1±2·06	75·6	82·6	88·8
Serum protein gm/100 ml. serum	8·9	5·1	6·79±0·25	8·61	..	..
Inorganic constituents						
Calcium mgm/100 ml. serum	11·4	9·4	10·5±0·10	10·4	10·5	11·1
Inorganic phosphorus mgm/100 ml. serum.	8·3	4·8	6·8±0·20	4·26	..	..
Magnesium phosphorus mgm/100 ml. serum.	3·10	1·71	2·35±0·11	2·78	2·40	2·40
Sodium phosphorus mgm/100 ml. serum.	480	253	3·69±15·24	..	..	..
Potassium phosphorus mgm/100 ml. serum.	16·5	11·9	14·0±0·28	..	..	..
Chlorides phosphorus mgm/100 ml. serum.	412	34	38·5±4·42	..	..	..

The range of blood sugar was from 70·6 to 102·3 with an average value of 88·1± 2·06 mg. per 100 ml. of blood. Mullick and Pal [*loc. cit.*] noted 88·3 and 82·6 mg. per 100 ml. of blood of Hariana and Dhanni bullocks respectively. While Kehar and Murty [*loc. cit.*] reported a lower value of 75·6 mg. per 100 ml. of blood for Hariana bullocks.

Serum proteins. These were estimated by the well known Kjeldahl method as described by Hawk *et al.*, [*loc. cit.*]. The mean serum protein content was 6·79±0·25 gm. per 100 ml. of serum, with a range of 5·1 to 8·9 Kehar and Murty [*loc. cit.*] reported an average value of 8·61 gm. per 100 ml. of serum, a value definitely higher than for the Kumaoni bullocks reported here.

Inorganic constituents

Calcium. Calcium was estimated by the method of Clark and Collip [1925]. The average calcium content of Kumaoni bullocks is 10·5±0·10 within the limits of 9·4 and 11·4 mg. per 100 ml. of serum and is in agreement with the calcium content of Hariana bullocks reported by Kehar and Murty [*loc. cit.*] and Mullick and Pal [*loc. cit.*]. The value of 11·1 mg. per 100 ml. of serum observed in Dhanni bullocks by Mullick and Pal [*loc. cit.*] is higher than the one recorded here for the Kumaoni bullocks.

Inorganic phosphorus. This constituent was estimated by the method of Fiske and Subbarow [1925] as described by Hawk *et al* [*loc. cit.*]. The mean inorganic

phosphorus content is 6·8±0·20 within the limits of 4·8 and 8·3 mg. per 100 ml. of blood sera. The figure reported for Hariana bullocks by Kehar and Murty [*loc. cit.*] is significantly lower.

Magnesium. Magnesium was estimated by the method of Fiske and Subbarow [*loc. cit.*] as described by Hawk *et al.*, [*loc.cit.*]. The mean magnesium content of the blood serum of Kumaoni bullocks is 2·35±0·11 with a range of 1·71 to 3·19 mg. per 100 ml. Mullick and Pal [*loc. cit.*] observed a similar value for the magnesium content of Hariana bullocks, but Kehar and Murty [*loc.cit.*] recorded a higher value, 2·78 for this breed. The Kumaoni bullocks exhibited a lower magnesium content than the Dhanni bullocks. [Mullick and Pal, *loc. cit.*]

Sodium. The method of Weinbach [1935] was used in estimating the sodium content of serum. A very wide range of 253 to 489 mg. per 100 ml. was noticed with an average amount of 369±15·24.

Potassium. The serum potassium was determined by the method of Breh and Gaebler [1930]. The maximum and minimum values were 16·5 and 11·5 mg. with an average of 14·0±0·28 mg. per 100 ml. of blood serum.

Chlorides. The serum chlorides were estimated by the method of Whitehorn [1920-21]. The average chloride content was 385±4·42 within the range of 344 to 412 mg. per 100 ml. of serum.

From the foregoing data, it is observed that the blood composition of Kumaoni bullocks, which showed lower haematological values as compared to those of pure bred cattle, compare favourably with the blood picture of buffaloes reported by Kehar and Murty [1951]. In view of the breed differences, in the haemoglobin concentration and the red cell count, observed by Manresa and Reyes [1934] and Mullick and Pal [1943], the low values recorded, for these constituents, in the present study, seems to be characteristic of the breed. The mean corpuscular volume and the mean corpuscular haemoglobin of goat blood [Murty and Kehar 1951] are lower than that of Kumaoni bullocks. The serum calcium and magnesium contents of Kumaoni bullocks are almost the same as observed in buffalo [Kehar and Murty, *loc. cit.*], goat [Murty and Kehar, *loc. cit.*] and pure bred cattle [Kehar and Murty, *loc. cit.*, Mullick and Pal, *loc. cit.*]. The serum calcium content is higher and the chlorides lower in equine blood [Kehar *et al*, 1940] as compared to that of Kumaoni bullocks.

It is observed that the Kumaoni bullocks recorded higher amounts of serum inorganic phosphorus and blood sugar but lower serum proteins, than the pure bred cattle, but they compare favourably with buffalo [Kehar and Murty, *loc. cit.*] and goat [Murty and Kehar, *loc. cit.*]. The sodium and chloride contents of goat serum [Kehar and Murty, *loc. cit.*] are lower and the potassium content similar to Kumaoni bullocks. The above findings indicate that not only the blood constituents of different species of domestic animals differ, but variations are also found in different breeds of the same species.

Summary

Previous investigations carried out in this laboratory had indicated that there may be a possibility of variations in the amounts of the constituents of the blood in

different breeds of cattle. Since Kumaoni bullocks are extensively used for metabolic and physiological experiments, attempts were made to establish the physiological standards for the blood constituents of this breed of cattle. These observations included the study of the morphological, organic and inorganic constituents of their blood. The average values with standard error are given below:

Haemoglobin: $7{\cdot}40 \pm 0{\cdot}18$ gms. per 100 ml. of blood; Red blood cell count $6{\cdot}50 \pm 0{\cdot}24$ millions and white cell count $8{\cdot}40 \pm 0{\cdot}33$ thousands per cubic millimeter of blood; cell volume—$35{\cdot}3 \pm 1{\cdot}34$ per cent; mean corpuscular volume—54·6. 1·17 cubic microns; mean corpuscular haemoglobin $11{\cdot}6 \pm 0{\cdot}29$ micro micro grammes mean corpuscular haemoglobin concentration $21{\cdot}2 \pm 0{\cdot}40$ per cent; sugar $88{\cdot}1 \pm 2{\cdot}06$ mgm. per 100 ml. of blood; protein $6{\cdot}79 \pm 0{\cdot}25$ gm., calcium $10{\cdot}5 \pm 0{\cdot}10$ mg., Inorganic phosphorus $6{\cdot}8 \pm 0{\cdot}20$ mg., magnesium $2{\cdot}35 \pm 0{\cdot}11$ mg., Sodium $369 \pm 15{\cdot}24$ mg.; Potassium $14{\cdot}0 \pm 0{\cdot}28$ mg., and chlorides $385 \pm 4{\cdot}42$ mg. per 100 ml. of blood serum.

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THE BUFFALO—A REVIEW

By E. S. E. Hafez, Ph.D. (Cantab), Faculty of Agriculture, Cairo, Egypt

(Received for publication on 13 March, 1952)

ONE of the most urgent problems facing milk production in the tropical and sub-tropical countries, is to find a suitable type of dairy animal which combines high milking capacity and tolerance to severe conditions of climate, disease and defective nutrition. The domestic buffalo is considered the premier milk producing animal in the tropics. Some of the characteristics of the buffalo render it more suitable than the cow for dairy farming in other parts of the world as well.

Zoological Status

The buffalo is a member of the ox-tribe, sub-family Bovinae. Five species of the wild buffalo (Table I) are known to have orginated in the equatorial and tropical zones in swampy moist districts of low altitudes. Their systematic classification is mainly based on the distribution of the hair on the body, the colour and size of ears and size of skulls. The characteristics of the horns are also considered, namely, their number (from 2 to 4), the degree of flatness, approximation to the forehead, direction, reflection, length and thickness of the base.

Table 1

Origin of wild buffalo (Sub-genus bubalus)

Latin name	English name	Origin	Number of varieties reported
Bos (Bubalus) *bubalis*, L.*	Indian	India and Assam	4
Bos (Bubalus) *caffer*, L.* or *Bos* (Syncerus) *caffer*, Sparrman†	African (Cape)	Algoa Bay, South Africa	21
Bos (Bubalus) *mindorensis*, L.*	Carabao	Mindoro and Philippine	1
Bos (Bubalus) *depressicornis*, L.*		Celebes, Asia	2
Bos (Bubalus) *pumilus*, L.‡		West Africa	..

*Lydekker [1913]
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Indian Buffalo (Bubalus bubalis)

The Indian buffalo has been domesticated since prehistoric times on the Indian plains, and it is not found at the present day in a truly wild state. The buffalo cow is met with in a more or less domesticated state throughout the north tropical and sub-tropical zones. It is found in India, a large portion of China, Malaya, Persia, Afghanistan, Bulgaria, Rumania and all the countries of the Mediterranean basin except France. In the southern tropical zone, it has been only introduced into Melville island near the northern coast of Australia. Thus different races of the domestic buffalo now exist and are essentially the same as the Indian buffalo with variation in size of horns, colour and texture of skin and slight difference in the conformation. Breeds have also been evolved, and in India there are over a dozen breeds, the most common reported by Kothavala [1935] are the Jaffarabadi, Murrah, Mehsana, Nagpuri and Surti.

The age of the domestic buffalo up to a certain age is indicated quite accurately by the teeth as in cattle. Its skin is thicker than that of cattle, the epidermis is thicker, the texture of the cornium is coarser, the hair and sweet glands are fewer and the skin secretion is less intense [Yamane and Ono, 1936]. Detailed studies on the growth of the buffalo as compared with that of cattle have been undertaken by Maymone [1945]. The number of chromosomes is 2n=48, forty rod-shaped and eight V-shaped, the V's being clearly distinguishable in size, while in cattle the chromosome number is 2n=60 [Makino 1944]. Cattle zebu crosses are fertile while cattle buffalo offspring have never been produced.

In their habits buffaloes resemble cattle apart from a few exceptions. They are strong swimmers (swim very low in the water only the eyes and nostrils above the surface) and are exceedingly fond of wallowing in pools, plastering themselves with mud, possibly to get rid of the external parasites. An account of their past, present and future in agriculture, and also a brief review on their reproduction follow.

(a) *Agricultural Utility.*—The buffalo is semi-aquatic in its natural habitat, and is to be found in places which, far from being swampy, provide severest climatic conditions both in the summer and in the winter, with a limited amount of rainfall. It is highly valued almost throughout the East wherever rice is cultivated. It can adapt itself to varying conditions to a remarkable degree. This is one of the main reasons why it does so well as a dairy animal in many countries where it has been imported under conditions different from those prevailing in its natural habitat.

In some countries, the buffalo is regarded as the best animal for butter production in the tropics, while cattle are superior for milk production. The buffalo is docile, hardy and a regular and steady yielder. It is not very susceptible to changes of place, milker or feed. The mammary glands are exceptionally well developed. Its capacity to convert feeding stuffs into milk is probably unequalled by any other species of farm animal. The lactation period lasts from 10 to 18 months and the milk contains nearly twice as much fat (buffalo's milk contains 6 to 13 per cent fat) and total solids as does the milk of the European cow. The buffalo's milk is used mainly for the production of butter (for table and cooking), while the by-products of the dairy industry (butter-milk, skim-milk and skim-cheese) are used for human and animal consumption. It has been stated that the application of water to the body surface of the animal lowers the body temperature and prevents reduction

in the milk-yield [Sinha and Minett, 1947]. The calf can be delivered without much difficulty. The buffalo calf may then be hand reared (pail suckling) with good attention.

Buffalo meat is thought to be coarse, but this is due to the fact that old animals are usually slaughtered, and the meat of an animal, four to six weeks old and properly fed, in no way differs from veal. With regard to the relative nutritive value of buffalo beef as compared with ox-beef and veal, it is stated that the former is distinctly superior in several respects. Buffalo beef differs markedly in structure from ox-beef, the muscle fibres being thicker and the nuclei more abundant. In transverse sections, the fibres are polygonal instead of irregularly shaped, the component fibrallae are larger and there is a greater amount of sarcoplasm. Differences also occur in regard to the distribution of the subcutaneous fat, connective tissue and elastic tissue.

The buffalo bull cannot stand cold or heat, yet he well adapts himself to hard work. In India, the buffalo is generally more susceptible than cattle to Foot-and-Mouth disease and Anthrax and the incidence of Anthrax is almost the same when compared to cattle.

Although some of the breeds of domestic buffalo have been introduced in a large number of places, their introduction in some sub-tropical countries is still limited. In the British colonies (tropical Africa) and in most parts of China there are large areas of swampy land which cannot be drained and which supply abundant grass on which the buffalo would no doubt be rendered productive. In the United States, the swamps and marshes of Florida, Louisiana and Mississippi should be especially suitable for buffalo production.

Selection must be made to ensure high milk yields from the dairy herds. More angular form, better development of the udder and milk veins may be attained by careful selection. The selection of the breeding animals may be based on the progeny testing of each individual buffalo-bull. Cross-breeding amongst the different breeds and races of the Indian buffalo might give desirable results, and artificial insemination could be applied on a larger scale. Further careful studies should be made before an attempt to spread the areas of buffalo raising.

(b) *Reproduction.*—It seems that the breeding season is continuous all the year round although conception occurs more frequently at certain periods of the year. In the Philippines, maximum sexual activity seems to occur between August and January [Villeges, 1928 ; Manresa and Diapo, 1938 ; Ocompo, 1939]. In India more frequent conceptions occur from August to November [Dave, 1938], while in Bulgaria the maximum ovarian activity coincides with the autumn and winter months [Kaleff, 1932]. In the Egyptian buffalo the average number of conception which required one, two and three services are 55·63 per cent, 21·62 per cent and 7·66 per cent, respectively while the average number of services per conception is $1{\cdot}46 \pm 0{\cdot}31$ [Hafez, 1952 a].

Conception followed by embryonic mortality is suggested for some of the animals which are served and do not conceive [Hafez, 1952 a]. In the Philippines, the buffalocow reaches puberty at the age of 26 to 29 months [Villeges, 1930] while in Egypt, the first oestrus is mainfested at the age of 15 to 18 months [Hafez, 1952 b].

The signs of oestrus and the behaviour of both sexes at mating have been described by Ocompo [1939], who has stated that the symptoms are more marked in the Philippine carabao than in the Indian. He has attributed the absence of the thick vaginal discharge during the heat period to the frequent urination of the oestrous female. In general, the symptoms seem to be much intense than in cattle. The duration of oestrus is about 1 or 1½ days and the maximum duration is four to five days as recorded by Ocompo [1939]. The length of the oestrous cycle, which is about 21 days, is more variable than that of the cow. The cycle length of 37 days reported by Ocompo [1939] may not actually be the length of one oestrous cycle but perhaps two cycles intervened by one silent heat. The histology of the vaginal mucosa does not denote the phase of the cycle [Manresa and Diapo, 1938]. The occurrence of ovulation does not depend on the age, the milk yield or the physical work; and in healthy animals as a rule conception follows ovulation [Kaleff, 1942]. Polding and Lall [1945] have given a descriptive account of the comparative genital anatomy (comparative measurements of genitalia) of the buffalo and the cow. They have stated that in the buffalo, the recent corpus lutem is veined with red and has a haemorrhagic protrusion (luteal protrusions 3 to 10 mm. in diameter) while the regressing corpora lutea are not veined and white in colour. They have also shown that never during the cycle is the luteal substance yellow while the luteal cavities (3 to 5 mm. in diameter) are common.

Symptoms of pregnancy and approaching parturition (changes in the genitalia, udder and vaginal discharges), behaviour during *post-partum* and the changes in the live weight and the body measurements of the pregnant buffalo-cow have been described by Ocompo [1939]. In Egyptian buffaloes, the average length of gestation is $316{\cdot}77 \pm 8{\cdot}8$ days and male calves are carried for a longer period than females [Ragab and Asker, 1951]. Maymone [1945], however, states that pergnancy is shorter in the case of the male foetus. The month of calving has a significant effect on length of gestation while the calving sequence of the dam or her age have no effect on the gestation period [Ragab and Asker, 1951]. Pregnancy is slightly shorter in the case of the pregnant heifer [Kaleff, 1932; Markus, 1943]. In general, the gestation period varies with the race as it does in cattle and it ranges from 287 to 340 days (Table II).

The interval between calving and *post-partum* heat seems to depend on genetic factors which are characteristic of the strains as well as the individuals [Kaleff, 1942]. Asdell [1946] has classified the Indian (Asiatic) buffalo into two races, a swamp race with nocturnal mating and with the *post-partum* heat occurring after 65 days. The other, a river race, mates during the day. In Bulgaria, buffaloes calving yearly come on heat at the beginning of their lactation period up to 118 days *post-partum*, while those calving every 1½ to 2 years show the *post-partum* heat at the end of the lactation period (8 to 12 months) [Kaleff, 1932]. The Egyptian buffalo-cow shows *post-partum* oestrus 22 days after calving, while conception requires from 1 to 4 services. This is due to the unprepared *post-partum* endometrium for embroy implantation [Hafez, 1952 a]. Table II summarizes the literature on the reproduction of the domestic buffalo in its different breeding centres.

As the gestation period is about 11 months, the calving season coincides with the period of the maximum ovarian activity. In Italy, the primiparous buffaloes calve from March to April while the multiparous ones calve from August to Sepetmber

TABLE II

Literature on the reproduction of the domestic buffalo (Indian buffalo and allied races)

Race	Cycle length (Days)		Duration of Oestrus (Hrs.)	Gestation period (Days)		Age at 1st conception (month)	Interval between calving and *post-partum* heat (Days)		Interval between calving (Days)		Authority
	Range	Mean		Range	Mean		Range	Mean	Range	Mean	
Bulgarian	12—28	21	24—34	303—326	315	..	14—410	118	..	..	Kaleff [1938]
Egyptian	..	21	12—36	..	..	15—18	10—76	35	..	..	Hafez [1952 a and b]
Indian	28—30	21	..	310—314	..	..	..	30	..	..	Levine [1943]
Indian (Surti)	..	..	..	299—325	313	36	..	..	..	..	Dave [1930]
Philippine	28—46	34	24			33	24—50	35	367—479	415	
Indian	34—39	37	24	314—320	316	46	45—53	50	488—582	520	Ocampo [1939]
Philippine × Indian	20—39	31	24			26	35—51	44	388—679	502	
Italian	..	..	..	287—337	311	..	..	..	..	..	Maymone [1945]
Malayan	30	..	..	330—340	..	28	30—60	..	..	480	Federated Malaya States [1920] Kangaratnan [1935]

[Maymone, 1945]. The number of calves in the lifetime of the buffalo-cow may reach 14 or 15 [Dave, 1940]. Genital abnormalities such as cystic ovaries, cystic corpora, dermoids, thin walled luteal bodies and the presence of cysts on the genitalia have been described by Polding and Lall [1945].

It would be of interest to find out the relationship of the interval between calvings to the milking production. The effect of age on the occurrence of ovulation and conception is important in determining the best age for culling the old animals from the breeding herds. An effort is needed to evaluate the importance of early foetal or embroyonic death followed by unseen abortion or resorbtion. It is suggested that the effect of physiological, pathological, genetic and nutritional functions may be studied on the reproductive efficiency of the buffalo-cow. This will help in a more adequate assessment of fertility than by the number of services required per conception or the percentage of pregnancies.

The buffalo-bull can be used up to 10 to 15 years and can serve 50 buffalo-cows per annum [Dave, 1940 ; Marsh and Dawson, 1948]. The buffalo semen is milk white in colour with a very light bluish tinge (the bull's semen has a more or less yellowish tinge). The average volume of the ejaculate is $3{\cdot}45 \pm 1{\cdot}40$ c.c. while the sediment (after centrifuging) varied from 8·5 to 25 per cent [Mahmoud, 1949]. The sperm count ranges from 210×106 to 2000×106 per c.c. while pH ranges from 6·6 to 7 and the most suitable storage temperature is 7°C [Mahmoud, 1949]. The former authority was able to differentiate between buffalo and bull semen by biometric, morphological and cytological evidence. Seasonal variation in the semen quality of the buffalo-bull has been stated by Badr-Eldin [1952]. Penicillin and sulphanilamide solutions have been successfully used to dilute buffalo semen to inhibit the growth of the bacterial contaminants [Mahmoud, 1952].

African Buffalo

The two species of wild buffalo found in Africa have never been found domesticable. Both are found rarely in remote districts where they live on aquatic plants. They are among the African game animals that have suffered most severely from rinderpest, especially the severe attack of 1890. At present the wild buffalo is found in the bushy, forest-clad districts of Cape Colony, Zululand, South-West Rhodesia and Portuguese East Africa [Haagner, 1920 ; Shortridge, 1934].

The evidence regarding their breeding season is somewhat conflicting, but suggests a fairly prolonged breeding season which depends on the climatic conditions. In South Africa, the breeding season appears to be from September to March, i.e. the spring and summer and the gestation period is some 11 months [Shortridge, 1934].

Summary

The Indian buffalo (*Bubalus bubalis*) has been domesticated and is found in many tropical and sub-tropical countries while the African buffalo (*Bos caffer*) is not domesticable. The buffalo is the best tropical source of butter as well as an important source of meat. It breeds all the year round, oestrus lasts for one day and the cycle length is 3 weeks. Pregnancy lasts 10½ months while the interval between calvings depends on factors such as heredity. Buffalo breeding should be

based on selection, progeny testing and crossbreeding although critical data on its productive and reproductive capacities is still lacking.

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CAUSES OF VARIATION IN BIRTH WEIGHT OF EGYPTIAN CATTLE AND BUFFALOES

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THE weight of an animal at any stage of life is a phenotypic expression of its genetype. The wider the difference between the two expressions, the less important is the phenotype as an aid to selection. The part played by environment in the determination of birth weight seems to be of special importance. Birth weight is the first measure of the prospective value of the animal's weight and it is more economical to cull the surplus males in the herd as early as possible. In considering the birth weight of animals in breeding plans, a full account of the importance of the different factors causing variation should be known.

This investigation aims at the study of how birth weight in Egyptian cattle, Pure-bred Shorthorn, Shorthorn grades and buffaloes is affected by genetical and non-genetical factors. The effect of sex, sequence of calving, month of calving and the sire of the calf were studied. Also the repeatability and heritability of birth weight were determined.

Material and methods of analysis

Birth weights of calves from cattle and buffaloes were available on 133 males and 153 females in the case of buffaloes and 140 males and 130 females in the case of cows, besides 133 calves of pure and graded Shorthorn of both sexes. All those calves were born as singles. The calves were born from 1930 to 1950 on the farm of the Faculty of Agriculture, Giza, Egypt. They were dropped mainly during Autumn, Winter and Spring; and few cases happened to be born in Summer. The buffalo calves were sired by seven bulls, while the cattle calves had six sires. Each bull had seven or more calves. Calves were usually weighed within 24 hours of their birth and their weights were recorded in kilogrammes.

The analysis of variance for sex and sequence of calving was worked out using the method suggested by Snedecor and Cox [1935]. In estimating the heritability of birth weight, the parental half-sibs method suggested by Lush [1948] was used.

Results and discussion

Effect of sex and sequence of calving—

The numbers and means of birth weights for cattle and buffaloes are presented in tables I and II respectively. Tables III and IV show the analysis of variance for the effect of sex and calving sequence on birth weight for Egyptian cattle and

6

buffaloes in the same order. The results show that females are lighter than male calves at birth in both cows and buffaloes. The difference between the two sexes is highly significant in both cases. This is in accordance with the findings of Knapp *et al* [1941] and Tyler *et al* [1947].

The average birth weight of buffalo calves was 38·50 and 36·41 kilos for males and females respectively. In the case of Egyptian cattle the averages were 25·77 and 23·93 kilos in the same order.

Buffaloes in both sexes exceeded cattle in birth weight by 12·73 and 12·83 kilos in the case of males and females. Such differences are expected since we are dealing with different species of animals.

TABLE I

Numbers and means of birth weight in kilos according to sex of the calf and calving, sequence of dam for Egyptian cattle.

Sequence	Males			Females		
	Number	Per cent	Mean	Number	Per cent	Mean
1st	28	20·00	23·75	26	20·00	21·73
2nd	41	29·29	25·95	30	23·08	23·40
3rd	25	17·86	26·60	25	19·23	24·00
4th	19	13·57	26·37	12	9·23	25·58
5th & 6th	16	11·43	26·81	19	14·61	25·37
7th & over	11	7·86	25·81	18	13·85	25·33
Total and Average	140	100·00	25·77	130	100·00	23·93

TABLE II

Numbers and means of birth weight in kilos according to sex of the calf and calving, sequence of dam for Egyptian buffaloes

Sequence	Males			Females		
	Number	Per cent	Mean	Number	Per cent	Mean
1st	24	18·05	36·13	39	25·49	33·67
2nd	25	18·80	36·40	24	15·69	34·96
3rd	30	22·55	39·23	26	16·99	36·96
4th	17	12·78	41·82	26	16·99	37·19
5th	10	7·52	39·20	20	13·07	38·15
6th & over	27	20·30	39·37	18	11·77	40·39
Total and Average	133	100·00	38·50	153	100·00	36·41

When dams get older, the average birth weight of their calves tends to increase. It is more pronounced in the early parturitions, *i.e.*, 1st, 2nd and 3rd ones in both cases, than it is in the later ones (Tables I and II). This may be due to the increase in the dams' weights as they advance in age and approach maturity. Moreover, the analysis of variance set out in Tables III and IV, showed that the mean square for sequence of calving is highly significant. The interaction between sex and sequence is not significant in either cows or buffaloes.

This result does not fully agree with other investigations carried out in this respect. Knapp *et al* [1941] stated that the first calves were lighter than their subsequents in birth weight, but beyond that parturition no consistent trend was evident. On the other hand Tyler *et al* [1947] reported that calving sequence has no influence on birth weight of calves after the second calving. Probably, the different methods used for raising dairy calves as well as the feeding and management of dairy heifers are responsible for the discrepancies between the results obtained in this subject.

TABLE III

Analysis of variance of birth weight for sex and calving, sequence in Egyptian cattle

Source of variation	Sum of squares	D. F.	Mean squares	Significance
Between sexes	275·46	1	275·46	H.S.
Between Calv. Seq.	393·72	5	78·74	H.S.
Sex. Sequence	45·68	5	9·13	N.S.
Within individuals	3,473·00	358	13·46	—

TABLE IV

Analysis of variance of birth weight for sex and calving sequence in buffaloes

Source of variation	Sum of squares	D. F.	Mean squares	Significance
Between sexes	252·36	1	252·36	Sig.
Between Calv. Seq.	1,014·53	5	202·91	H.S.
Sex Sequences	162·33	5	32·47	N.S.
Within individuals	11,061·82	274	40·37	—

TABLE V

Analysis of variance of birth weight for sires (Egyptian cattle)

Source of variation	Sum of squares	D. F.	Mean squares
Between sires	312·65	6	52·11**
Within ,,	2,379·33	191	12·46
Total	2,691·98	197	..

TABLE VI

Analysis of variance of birth weight for sires (buffaloes)

Source of variation	Sum of squares	D. F.	Mean squares
Between sires	1,251·95	5	250·39**
Within ,,	7,901·73	222	35·59
Total	9,153·68	227	..

** Highly significant=<·01

Effect of sire and heritability of birth weight—

During the period of this investigation, seven bulls and six buffalo-bulls were used in cattle and buffalo herds. It is shown in Tables V and VI that the differences between sire groups in birth weight were highly significant. In other words, some sires tended to produce heavier calves while others produced lighter ones, than the average.

The same Tables V and VI were utilised in estimating the heritability of birth weight for cattle and buffaloes. The parental half-sib correlation method was used since birth weights of dams and sires were not available. Estimates for heritability of birth weight obtained, were ·417 and ·561 for cattle and buffaloes respectively.

Gregory *et al* [1950] reported an estimate of ·45 for the heritability of birth weight in beef cattle. When the same authors used smaller numbers they obtained an estimate of 1·00. They stated that their estimates may be questioned because of the small number of calves used to evaluate the sires. Tyler *et al* [1947] reported that the average estimate of heritability for birth weight was 60 per cent, using

different correlations between relatives. Knapp and Nordskog [1946] and Dawson *et al* [1947] used sire-offspring regression and parental half-sibs methods for estimating the heritability of birth weight in beef cattle. Their estimates ranged between 11 and 42 per cent.

This analysis shows that the heritability of birth weight of Egyptian cattle and buffaloes are higher than most of the previously mentioned results. Such results are expected since cattle and buffaloes in Egypt are mixed populations and consequently the genetic variability of birth weight should be higher than those of the established European breeds of cattle.

According to such high estimates of heritability for birth weight in both Egyptian cattle and buffaloes, it could be concluded that phenotypic selection could be used for improving birth weights of animals. Since the genetic relation between the birth weight and other subsequent weights was not investigated in this study, it seems difficult to depend only on such measure for increasing the weights of animals. However, Kadi [1952] working on two breeds of Egyptian sheep, found that the genetic correlation between the birth weight and weaning weight on the one hand, and birth weight and marketing weight on the other hand, were all near unity. If the case in buffaloes and cattle is proved to be the same, selection based on birth weight will not lead to any loss in the number of genes controlling mature weights.

TABLE VII

Correlation coefficients for birth weights of Egyptian cattle and buffaloes for various combinations of calving sequences

Comparisons made	Cattle		Buffaloes	
	Number	r.	Number	r.
I and II	30	—·099	24	·384
II and III	38	·277	28	·630
III and IV	33	—·029	39	·476
I and III	50	·285	15	·501
I and IV	15	·129	15	·692
II and IV	25	·071	22	·596
Total and average	191	·048	143	·548

Repeatability of birth weight—

The repeatability of birth weight for cattle and buffaloes is shown in Table VII. The calculations have been made for consecutive and non-consecutive calvings. The results obtained for buffaloes are not in agreement with those for cattle. The average correlation coefficient for buffaloes is ·548 while it is ·048 for cattle. Differences between sexes were not taken into consideration when estimates of repeatability were worked out. It would be expected to obtain higher values, had correction for sex been applied since there was a significant difference between the two sexes in birth weight.

Effect of month of calving—

Month of calving was proved to be of no effect on birth weight in both buffaloes and cattle (Tables VIII and IX). Calving occurred during the months from August to March. The numbers of parturitions during the months of April, May, June and July were too few to be of practical value. The average birth weight for buffalo calves born during the above mentioned months, ranged from 35·42 to 38·74 kilos. In Egyptian cattle the average birth weight ranged from 23·18 to 25·71 kilos. The difference between the mean weights in the different months was not significant.

The main effect of the month of calving would lie in that the seasons of the year differ in the kind of pasture, feeds and temperature. The first two factors are the most important in this aspect. Eckles [1919] stated that the ration fed to the dam had little effect on the birth weight of the calf. He believes, however, that in extreme cases there would be an effect; while Fitch *et al* [1924] reported that the feed given to the dam has some influence on birth weight but less than is commonly supposed. They suggest that an effect may be obtained on a very restricted diet.

TABLE VIII

Analysis of variance of birth weight for month of calving in Egyptian Cattle

Source of variation	Sum of squares	D. F.	Mean squares
Between months	113·43	7	16·20
Within ,,	2,937·68	244	12·04
Total	3,051·11	251	..

TABLE IX

Analysis of variance of birth weight for month of calving in buffaloes

Source of variation	Sum of squares	D. F.	Mean squares
Between months	336·20	7	48·00
Within ,,	11,957·67	271	44·12
Total	12,293·87	278	..

The herds of the Faculty were under a stable system of feeding for the whole period of study. Animals received their rations according to weight and to production. During Winter they were fed only on clover if it could suffice their needs, otherwise they were given a supplementary ration of concentrates. This system has been followed all through the long time of investigation without much change. Therefore, one could not expect that month of calving to be a source of variation in birth weight of calves under such stable system of feeding and management. However, the present results agree with the results obtained by Tyler *et al* [1947] and Knapp *et al* [1941].

Effect of grading—

Rhoad, Phillips and Dawson [1945] reported that when crosses are made between breeds, the sire often has a marked effect on the birth weight of the calf.

In this data, it was the aim of the study to ascertain whether the pure shorthorn calves differ significantly from the shorthorn grades as far as the birth weight is concerned. The average birth weights of pure-bred, one half, three quarters and seven eighth Shorthorn were 30·82, 30·32, 32·30 and 31·00 kilos respectively. It is noticed that the average birth weight of half bred animals was very close to that of pure shorthorn. It was expected that according to the multiple factor theory the birth weight of half bred should lie between that of the parents, *i.e.*, Egyptian cattle and pure Shorthorn. The fact that heterosis is responsible for such increase is quite evident here. However, the analysis of variance in table X showed that there were no significant differences between birth weight of pure-bred Shorthorn and different grades.

This result denotes that improving birth weight of Egyptian cattle by changing the genotype of the calf through grading is attained in the first generation. Increasing the percentage of Shorthorn blood in the successive generations did not lead to any significant increase in birth weight. Moreover, the data showed that the mean birth weights of different grades of Shorthorn were heavier than that of native cattle. The difference between the two groups in the birth weight was 6·58 kilos.

TABLE X

Analysis of variance of birth weight for different grades of shorthorn

Source of variation	Sum of squares	D. F.	Mean squares
Between groups	77·67	3	25·89
Within ,,	2,736·97	130	21·05
Total	2,814·64	133	..

Summary

A study was carried out on the influence of the sex of the calf, calving sequence, month of calving and sire of the calf on birth weight of Egyptian cattle and buffaloes. The heritability as well as the repeatability of birth weight in both species were estimated. The effect of grading Egyptian cattle with pure-bred Shorthorn on birth weight of calves was determined. The study covered a period of twenty years (1930-1950) and included 286, 270 and 133 calves of both sexes from buffaloes, native cattle and pure-bred Shorthorn and grades respectively.

The average birth weight of male calves was 38·50 kilos in the case of buffaloes, while the mean birth weight of females was 36·41 kilos. In the case of Egyptian cattle, the mean birth weights were 25·77 and 23·93 kilos for males and females respectively. The differences between sexes in cattle and buffaloes were significant.

Sequence of calving was responsible for bringing about significant differences between birth weights of calves in cattle and buffaloes. Month of calving appeared to have no effect on birth weight of calves.

The heritability estimate for birth weight in buffaloes was 0·561 compared to 0·417 for cattle. The repeatability of birth weight was ·548 and 0·048 for buffaloes and cattle respectively.

Grading Egyptian cattle with purebred Shorthorns caused an increase in the birth weight of calves. The difference between grades was not significant, also grade Shorthorns were 6·6 kilos heavier in their birth weight than Egyptian calves.

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REGIONAL DISTRIBUTION OF *TABANUS* FLIES IN INDIA AND ITS RELATIONSHIP TO THE INCIDENCE OF SURRA

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(With Plates XX-XXI)

THE flies belonging to the genus *Tabanus*, commonly known as horse-flies or gnats, are widely prevalent in India, having been recorded from tropical, sub-tropical, and temperate regions. The adults are strong fliers and occur abundantly in the neighbourhood of water both on the plains and hills. All the common species are blood suckers and attack warm blooded animals including man. Of the diseases transmitted by these flies, surra is the most important, affecting horses, mules, camels, cattle, etc. These flies lay their eggs generally on leaves over-hanging water. After hatching the larvae fall into the water and develop. When mature, they crawl to dry soil, pupate and after a few days emerge as adult flies.

The available meagre, scattered data on the distribution of *Tabanus* in India comprise the catalogue by Senior-White [1927] and the records based on the specimens received from field workers for identification at this Institute. In order to provide an up-to-date ready reference to the distribution of species of *Tabanus* in India, their occurrence, compiled from various sources has been recorded in this paper. The study on the distribution of these flies has been extended as far as possible to Pakistan and other surrounding countries as a knowledge of their occurrence in the neighbouring countries will be useful in devising control measures. For this purpose the division of the country into five regions on the basis of topography, climate, rainfall and vegetation, etc., as recommended by the Indian Council of Agricultural Research for the orientation of agricultural research, has been adopted. Their incidence has also been discussed according to four natural physical regions of the country, *viz.*, montane, submontane, pastural, and coastal.

Region 1—

Temperate Himalayan Region.—It comprises Kashmir, hilly tracts of Uttar Pradesh, Punjab, Bengal, and Assam. This region covers the Himalayan ranges interspersed with extensive ravines clothed with dense forests of a tropical type at low elevations and of a temperate type higher up. Certain tracts have a heavy rainfall, 120 inches or more, with the humidity varying considerably in the different seasons. Though the majority of the hill streams are fast running, there are several sluggish streams, rain pools and innumerable seepage areas and side pockets along the river banks.

This region contains also the submontane zone, *i.e.*, the foot hills, with a very heavy rainfall, up to 220 inches, mostly distributed between the months of June and September. The land has a slope and the soil is generally porous. It is traversed by numerous rivers, streams and seepages of varying sizes particularly during the rains. The local rainfall has very little effect on them for unless the sub-soil water on the hills is high the streams do not have water for more than a day even if there has been heavy downpour locally.

The places described above are ideal for the breeding of *Tabanus* flies as is evident from the fact that nineteen species have been recorded from this region. They are as follows :—

1. *T. diversifrons* Ricardo
2. *T. explicatus* Walker
3. *T. fulvimedius* Walker
4. *T. fuscomaculatus* Ricardo
5. *T. hirtipalpis* Ricardo
6. *T. hirtus* Walker
7. *T. jucundus* Walker
8. *T. leleani* Austen
9. *T. leucocnematus* Bigot
10. *T. leucopogon* Bigot
11. *T. orientis* Walker
12. *T. oxyceratus* Bigot
13. *T. provincialis* Ricardo
14. *T. rubidus* Wiedemann
15. *T. sexcinctus* Ricardo
16. *T. striatus* Fabricius
17. *T. subcallosus* Ricardo
18. *T. tropicus* Panzer
19. *T. wyvillei* Ricardo

From the range of distribution of the above nineteen species, it is seen that seven species, *viz.* (1) *T. fulvimedius*, (2) *T. hirtipalpis*, (3) *T. hirtus*, (4) *T. leucopogon*, (5) *T. oxyceratus*, (6) *T. tropicus*, and (7) *T. wyvillei* are exclusive to this region while the remaining twelve extend beyond it and have been recorded from one or more other regions, *vide* Table I.

Region 2—

Eastern Wet Region.—It comprises the States of Assam, Bengal, Bihar, Orissa, and the eastern parts of Uttar Pradesh, Vindhya Pradesh, Madhya Pradesh, and Mardrs. It has a rainfall of 50 inches or above and is predominantly rice growing. The area mostly falls within the Gangetic basin though certain coastal alluvium are also included.

Physical features of this region are not entirely uniform. In some areas the land is dry with a porous soil consisting largely of a ferruginous laterite gravel, sand and clay or composed of an old alluvium, while others are composed entirely of recent alluvium, some of lime stone with a superficial layer of alluvium. There are also undulating areas consisting of barren rocky country traversed by numerous small streams. Some areas are devoid of large trees and the vast stretches of land are covered with jungle with dwarf trees and grassy wastes usually above the flood level. There are some zones within the limit of floods, composed entirely of recent alluvium traversed by a number of distributaries of the Ganga and other rivers some of which are alive while others are in a moribund condition. There is yet another zone in this region which may be termed coastal, comprised of very low lying lands subject to flooding by tides. This area is clothed with a dense tidal forest mostly of mangrove type.

Table I

Showing the incidence of different species of *Tabanus* in various regions of India and the surrounding countries

Different regions of India and surrounding countries								
I	II	III	IV	V	Ceylon	Burma	Perso-Baluchi Afghanistan	Nicobar Islands
						1		
							2	
	4							
					5			
					6			
	7							
			8					
		9	9					
	10					10		
	11	11	11	11		11		
					12			
	13							
				14				
				15				
					16			
	17	17	17	17				
18	18							
		20					17	
21			21					
	22	22	22				19	
					23			
	24				24	24		
25						25		
					26	26		
					27			
28	28					28		

TABLE I—*contd.*

Showing the incidence of different species of *Tabanus* in various regions of India and the surrounding countries—*contd.*

Different regions of India and surrounding countries								
I	II	III	IV	V	Ceylon	Burma	Perso-Baluchi Afghanistan	Nicobar Islands
30								
						31		
32								
	33					33	29	
			34	34				
					35			
	36							
37		37	37		37			
						38		
	39	39						
40		40						
41	41		41			41		
			42					
43								
	44	44	44	44			40	
	45							
	46							
	47					47		
	48	48						
	49							
	52		52					50
	53							
54		54				54		
55								
56				56				
						55		
					58			

TABLE I—*contd.*

Showing the incidence of different species of *Tabanus* in various regions of India and the surrounding countries—*contd.*

Different regions of India and surrounding countries								
I	II	III	IV	V	Ceylon	Burma	Perso-Baluchi Afghanistan	Nicobar Islands
	59							
60	60	60	60	60	60	59	57	
	61		61			60		
62	62							
64	63	64	63	63	63			
65	64	65	64					
	67		67			65		
						66		
		68						
			69					
	70	70	70	70	70	70		
72								
	73		73					
	74	74	74					
75								

The following three species have also been recorded from India, but their exact localities are not known.

3 *T. albocostatus* Bigot
51 *T. nigropictus* Macquart
71 *T. trinominatus* Senior-White

This region is further characterised by the presence of borrow pits, brick fields, railway side ditches and dead channels, etc., all providing ideal breeding places for tabanid flies. In this region *Tabanus* is represented by thirty-two species, namely :—

1. *T. albofasciatus* Ricardo
2. *T. auriflamma* Walker
3. *T. birmanicus* Bigot
4. *T. brunnipennis* Ricardo
5. *T. conicus* Bigot
6. *T. ditaeniatus* Macquart
7. *T. diversifrons* Ricardo
8. *T. flavicinctus* Ricardo

9. *T. flaviventris* Bigot
10. *T. fuscomaculatus* Ricardo
11. *T. hybridus* Wiedemann
12. *T. joidus* Bigot
13. *T. khasiensis* Senior-White
14. *T. leucocnematus* Bigot
15. *T. macer* Bigot
16. *T. manipurensis* Bicords
17. *T. melanognathus* Bigot
18. *T. monotaeniatus* Bigot
19. *T. nemocallosus* Ricardo
20. *T. nephodes* Bigot
21. *T. obconicus* Walker
22. *T. obtatus* Walker
23. *T. rubicundus Macquart*
24. *T. rubidus* Wiedemann
25. *T. rufiventris* Fabricius
26. *T. sexcinctus* Ricardo
27. *T. speciosus* Ricardo
28. *T. striatus* Fabricius
29. *T. subhirtus* Ricardo
30. *T. tenens* Walker
31. *T. tuberculatus* Ricardo
32. *T. virgo* Wiedemann

The following nine species (1) *T. albofasciatus* (2) *T. auriflamma* (3) *T. conicus* (4) *T. hybridus* (5) *T. joidus* (6) *T. manipurensis* (7) *T. melanognathus* (8) *T. nephodes* (9) *T. tropicus* are specific to this region while others have crossed the regional boundary, *vide* Table I.

Region 3—

North Western Dry Region.—This region comprises parts of Jammu and Kashmir, Western Pakistan, Eastern Punjab, Patiala and East Punjab States Union, Greater Rajasthan, Western Uttar Pradesh, Madhya Bharat, and Delhi. The area is mostly dry with an average rainfall of thirty inches or less. In the north-west lies the great Rajputana desert with an average rainfall of less than five inches. The rivers of the Punjab as also the Ganga and its northern tributaries run through parts of this region which lies mostly in the Indus valley and is predominantly wheat growing. Consequent upon the introduction of extensive canals system wide marshy areas with concomitant growth of vegetation have developed providing ideal breeding sites for *Tabanus* flies. *Tabanus* is represented in this region by seventeen species, namely :—

1. *T. bicinctus* Ricardo
2. *T. brunnipennis* Ricardo
3. *T. ditaeniatus* Macquart
4. *T. excelsus* Ricardo
5. *T. flavicinctus* Ricardo
6. *T. jucundus* Walker
7. *T. khasiensis* Senior-White
8. *T. leleani* Austin
9. *T. macer* Bigot
10. *T. Nemocillosu* Ricardo
11. *T. orientis* Walher
12. *T. rubidus* Wiedemann
13. *T. striatus* Fabricius
14. *T. subcallosus* Ricardo
15. *T. sufis* Jaennicke
16. *T. tenens* Walker
17. *T. virgo* Wiedemann

Only two of these, *viz. T. excelsus* and *T. sufis* are confined to this region, the remaining fifteen species occur in one or more of the other regions also.

Region 4—

Southern Region.—This region comprises mostly the peninsular area consisting of Madhya Bharat, parts of Madhya Pradesh and Vindhya Pradesh, Hyderabad, Mysore, Coorg, Sourashtra, Cutch, and greater parts of Madras and Bombay. The

area has an average rainfall of twenty-five to fifty inches and is largely millet growing. It is a roughly triangular undulating land of moderate elevation interespersed with tracts of somewhat higher forest-covered hills and plateaus. In parts, the land is very fertile and there are many areas of forest specially to the north and the west.

The central plateau as a whole slopes to the east and almost all the rivers flow from west to east. Of these, the most important are the Mahandai draining the north-west, the Godavari and Krishna draining the central portion of the Deccan and the Pennar and Cauvery in the south. In the north, however, the rivers Tapti and Narbadda flow in the opposite direction and, passing on either side of the Satpura range, enter the gulf of Cambay. Most of these rivers form deltas at their mouth, which provide ideal breeding places for the tabanid flies. From these rivers a few irrigation projects have been developed in the south raising the subsoil water level which along with numerous ponds, borrow pits, and a moderately heavy rainfall provide innumerable breeding places for these flies.

In this region twenty-one species of *Tabanus* are found, namely :—

(1) *T. auristriatus* Ricardo
(2) *T. bicinctus* Ricardo
(3) *T. brunnipennis* Ricardo
(4) *T. ditaeniatus* Ricardo
(5) *T. expeicatus* Walker
(6) *T. flavicinctus* Ricardo
(7) *T. indianus* Ricardo
(8) *T. jucundus* Walker
(9) T. *leucocnematus* Bigot
(10) *T. leucohirtus* Ricardo
(11) *T. macer* Bigot
(12) *T. obconicus* Walker
(13) *T. rubidus* Wiedemann
(14) *T. rufiventris* Fabricius
(15) *T. speciosus Ricardo*
(16) *T. striatus Fabricius*
(17) *T. subhirtus* Ricardo
(18) *T. tenebrosus* Walker
(19) *T. tenens* Walker
(20) *T. tuberculatus* Ricardo
(21) *T. virgo* Wiedemann

There are only three species, namely, (1) *T. tenebrosus* (2) *T. leucohirtus* and (3) *T. auristriatus* which are exclusive to this region whereas the remaining eighteen species have been reported from one or more other regions, *vide* Table I.

Region 5—

South Coastal Wet Region.—This region comprises the eastern coastal region of Madras, the western coasts of Madras and Bombay States and the United States of Travancore and Cochin. It is a narrow fringe of low lying coastal land surrounding the southern plateau of peninsular India forming the eastern and western maritime plains, the latter in places being a mere fringe of land a few miles wide. In Malabar tract on the west coast, there are the high mountain ranges of the western ghats and

9. *T. flaviventris* Bigot
10. *T. fuscomaculatus* Ricardo
11. *T. hybridus* Wiedemann
12. *T. joidus* Bigot
13. *T. khasiensis* Senior-White
14. *T. leucocnematus* Bigot
15. *T. macer* Bigot
16. *T. manipurensis* Bicords
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18. *T. monotaeniatus* Bigot
19. *T. nemocallosus* Ricardo
20. *T. nephodes* Bigot
21. *T. obconicus* Walker
22. *T. obtatus* Walker
23. *T. rubicundus Macquart*
24. *T. rubidus* Wiedemann
25. *T. rufiventris* Fabricius
26. *T. sexcinctus* Ricardo
27. *T. speciosus* Ricardo
28. *T. striatus* Fabricius
29. *T. subhirtus* Ricardo
30. *T. tenens* Walker
31. *T. tuberculatus* Ricardo
32. *T. virgo* Wiedemann

The following nine species (1) *T. albofasciatus* (2) *T. auriflamma* (3) *T. conicus* (4) *T. hybridus* (5) *T. joidus* (6) *T. manipurensis* (7) *T. melanognathus* (8) *T. nephodes* (9) *T. tropicus* are specific to this region while others have crossed the regional boundary, *vide* Table I.

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1. *T. bicinctus* Ricardo
2. *T. brunnipennis* Ricardo
3. *T. ditaeniatus* Macquart
4. *T. excelsus* Ricardo
5. *T. flavicinctus* Ricardo
6. *T. jucundus* Walker
7. *T. khasiensis* Senior-White
8. *T. leleani* Austin
9. *T. macer* Bigot
10. *T. Nemocillosu* Ricardo
11. *T. orientis* Walher
12. *T. rubidus* Wiedemann
13. *T. striatus* Fabricius
14. *T. subcallosus* Ricardo
15. *T. sufis* Jaennicke
16. *T. tenens* Walker
17. *T. virgo* Wiedemann

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The central plateau as a whole slopes to the east and almost all the rivers flow from west to east. Of these, the most important are the Mahandai draining the north-west, the Godavari and Krishna draining the central portion of the Deccan and the Pennar and Cauvery in the south. In the north, however, the rivers Tapti and Narbadda flow in the opposite direction and, passing on either side of the Satpura range, enter the gulf of Cambay. Most of these rivers form deltas at their mouth, which provide ideal breeding places for the tabanid flies. From these rivers a few irrigation projects have been developed in the south raising the subsoil water level which along with numerous ponds, borrow pits, and a moderately heavy rainfall provide innumerable breeding places for these flies.

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(3) *T. brunnipennis* Ricardo
(4) *T. ditaeniatus* Ricardo
(5) *T. expeicatus* Walker
(6) *T. flavicinctus* Ricardo
(7) *T. indianus* Ricardo
(8) *T. jucundus* Walker
(9) T. *leucocnematus* Bigot
(10) *T. leucohirtus* Ricardo
(11) *T. macer* Bigot
(12) *T. obconicus* Walker
(13) *T. rubidus* Wiedemann
(14) *T. rufiventris* Fabricius
(15) *T. speciosus Ricardo*
(16) *T. striatus Fabricius*
(17) *T. subhirtus* Ricardo
(18) *T. tenebrosus* Walker
(19) *T. tenens* Walker
(20) *T. tuberculatus* Ricardo
(21) *T. virgo* Wiedemann

There are only three species, namely, (1) *T. tenebrosus* (2) *T. leucohirtus* and (3) *T. auristriatus* which are exclusive to this region whereas the remaining eighteen species have been reported from one or more other regions, *vide* Table I.

Region 5—

South Coastal Wet Region.—This region comprises the eastern coastal region of Madras, the western coasts of Madras and Bombay States and the United States of Travancore and Cochin. It is a narrow fringe of low lying coastal land surrounding the southern plateau of peninsular India forming the eastern and western maritime plains, the latter in places being a mere fringe of land a few miles wide. In Malabar tract on the west coast, there are the high mountain ranges of the western ghats and

the wet coastal area of the peninsula. Most of this part is covered with thick tropical forests though there are many areas near the coast which have been cleared for cultivation. The majority of the more important rivers flow from west to east forming deltas on the east coast, and afford suitable breeding sites for tabanid flies. The area has a heavy rainfall, receiving both the south-west and the north-east monsoons during which period innumerable small rivulets spring up and the land becomes water logged and provides ideal breeding places for *Tabanus*.

Ten species of *Tabanus* occur in the region, namely :—

(1) *T. brunnipennis* Ricardo
(2) *T. consanguineus* Macquart
(3) *T. demellonis* Senior-White
(4) *T. ditaeniatus* Macquart
(5) *T. indianus* Ricardo
(6) *T. macer Bigot*
(7) *T. provincialis* Ricardo
(8) *T. rubidus* Wiedemann
(9) *T. speciosus* Ricardo
(10) *T. tenens* Walker

Of the above, *T. consanguineus* and *T. demellonis* are specific to this region while the remaining eight are reported from one or more other regions (Table I).

Of the seventy-five species found in the above five regions (Plate XX, Table I), only *T. rubidus* Wiedemann is cosmopolitan. The species *T. albocostatus* Bigot, *T. nigropictus* Macquart, and *T. trinominatus* Senior-White have also been recorded from India but their exact geographical distribution in the country has not been mentioned.

The distribution of the fifty-four species of the tabanid flies found in the Indian Union has also been studied in relation to the natural physical divisions of the country, *i.e.*, Montane, Submontane, Pastural and Coastal, *vide* Table II. Of the thirty species recorded from the Montane region, ten are specific to it while the other twenty extend beyond its borders. In the Submontane region thirty-two species occur, of which three are specific to the region and the other twenty-nine are found elsewhere too. In the third region, namely Pastural, twenty-three species are represented of which only seven are exclusive to it. Fifteen species are found in the Coastal region though only three are confined to it. Only two species, namely *Tabanus rubidus* Wiedemann and *Tabanus striatus* Fabricius are cosmopolitan to all the four physical regions.

The distribution of insects and the diseases they transmit are not restricted by political boundaries and since a knowledge of the occurrence of vectors in the surrounding countries is essential in formulating control measures, these studies have been extended to the tabanid fauna of Ceylon, Burma, and Perso-Baluchistan.

Ceylon.—About three-fourths of the land along the north and the east resemble the main area of peninsular India and is almost plain or slightly undulating country of no great elevation with an average annual rainfall of about fifty inches. The rest of the southern part of Ceylon, like Malabar tract, is hilly with rich tropical forests and an average rainfall of over hundred inches.

EXPLANATION OF PLATE XX

KEY TO NUMBERS

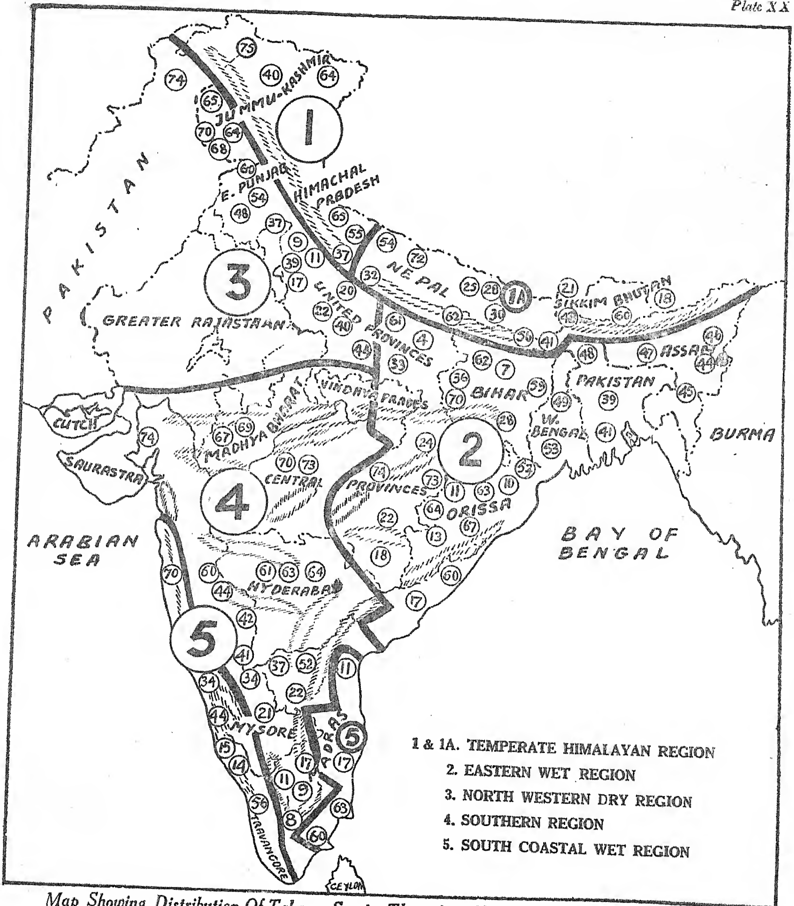
1 Tabanus absconditus
2 T. adjacens
3 T. albocostatus
4 T. albofasciatus
5 T. angustilimbatus
6 T. atrohirtus
7 T. auriflamma
8 T. auristriatus
9 T. bicinctus
10 T. birmanicus
11 T. brunnipennis
12 T. ceylonicus
13 T. conicus
14 T. consanguineus
15 T. demellonis
16 T. discrepans
17 T. diateniatus
18 T. diversifrons
19 T. eggeri
20 T. excelsus
21 T. explicatus
22 T. flavicinctus
23 T. flavissimus
24 T. flaviventris
25 T. fulvimedius
26 T. fuscicauda
27 T. fuscicrura
28 T. fuscomaculatus
29 T. glaber
30 T. hirtipalpis
31 T. hirtistriatus
32 T. hirtus
33 T. hybridus
34 T. indianus
35 T. indiscriminatus
36 T. joidus
37 T. jucundus
38 T. lakhyamensis
39 T. khasiensis
40 T. leleani
41 T. leucocnematus
42 T. leucohirtus
43 T. leucopogon
44 T. macer
45 T. manipurensis
46 T. melanognathus
47 T. monotaeniatus
48 T. nemocallosus
49 T. nephodes
50 T. nicobarensis
51 T. nigropictus
52 T. oloaniceps
53 T. optatus
54 T. orientis
55 T. oxyceratus
56 T. provincialis
57 T. pulchellus
58 T. puteus
59 T. rubicundus
60 T. rubidus
61 T. rufiventris
62 T. sexcinctus
63 T. speciosus
64 T. striatus
65 T. subcallosus
66 T. subcornetaceous
67 T. subhirtus
68 T. sufis
69 T. tenebrosus
70 T. tenens
71 T. trinominatus
72 T. tropicus
73 T. tuberculatus
74 T. virgo
75 T. wyvilled

Explanation of Plate XX

KEY TO NUMBERS

1 Tabanus abscondens
2 T. adjaceus
3 T. albocfstatus
4 T. albofasciatus
5 T. angustilimbatus
6 T. atrohirtus
7 T. auriflamma
8 T. auristriatus
9 T. bicinctus
10 T. birmanicus
11 T. brunnipennis
12 T. ceylonicus
13 T. conicus
14 T. consangvineus
15 T. demellonis
16 T. discrepans
17 T. diateniatus
18 T. diversifrons
19 T. eggeri
20 T. excelsus
21 T. explicatus
22 T. flavicinctus
23 T. flavissimus
24 T. flaviventris
25 T. fulvimediu
26 T. fuscicauda
27 T. fuscicrura
28 T. fuscomaculatus
29 T. glaber
30 T. hirtipalris
31 T. hirtistriatus
32 T. hirtus
33 T. hybridus
34 T. indianus
35 T. indiscriminatus
36 T. joidus
37 T. jucundvs
38 T. kakhyenensis
39 T. khasiensis

40 T. leleani
41 T. leucocnematus
42 T. leucohirtus
43 T. leucopogen
44 T. macer
45 T. manipurensis
46 T. melanognathus
47 T. monotaeniatus
48 T. nemocallosus
49 T. nephodes
50 T. nicibarensis
51 T. nigropictus
52 T. obconicus
53 T. optatus
54 T. orientis
55 T. oxyceratus
56 T. provincialis
57 T. pulchellus
58 T. puteus
59 T. rubicundus
60 T. rubidus
61 T. rvfiventris
62 T. sexcinctus
63 T. speciosus
64 T. striatus
65 T. subcallosus
66 T. subesnerascens
67 T. subhirtus
68 T. sufis
69 T. tenebrosus
70 T. tenens
71 T. trinominatus
72 T. tropicus
73 T. tuberculatus
74 T. virgo
75 T. wyvilled



Map Showing Distribution Of Tabanus Species Throughout The Different Regions of India and Adjacent Territories

TABLE II

Showing the distribution of *Tabanus* flies in the different physical divisions of India, *viz.* montane, sub-montane, pastural and coastal

Different divisions of India			
Montane	Sub-montane	Pastural	Coastal
4	—	—	—
—	—	6	—
7	7	—	—
8	8	—	—
9	9	—	—
10	—	—	—
—	11	11	—
—	—	—	13
—	—	—	14
—	—	—	15
—	17	17	17
18	18	—	18
—	20	—	—
21	—	—	—
22	22	—	—
—	24	24	24
25	—	—	—
28	28	—	—
30	—	—	—
32	—	—	—
—	33	33	—
—	34	34	34
—	36	—	—
37	37	—	—
39	—	39	—
40	40	—	—
41	41	—	—

TABLE II—*contd.*

Showing the distribution of *Tabanus* flies in the different physical divisions of India, *viz.* montane, sub-montane, pastural and coastal

Different divisions of India			
Montane	Sub-montane	Pastural	Coastal
—	42	42	—
43	—	—	—
—	44	44	44
—	45	—	—
—	—	46	—
47	47	—	—
—	—	48	—
49	49	—	—
—	—	52	—
—	—	53	—
54	54	—	—
55	—	—	—
56	56	—	—
59	59	—	—
60	60	60	60
61	61	—	—
62	—	—	—
—	—	63	63
64	64	64	64
65	65	65	—
—	—	67	67
—	—	68	—
—	—	69	—
—	70	70	70
72	72	—	—
—	73	73	73
—	74	74	74
75	—	—	—

So far, fourteen species of *Tabanus* have been recorded from Ceylon.

(1) *T. angustilimbatus* Senior-White
(2) *T. atrohirtus* Ricardo
(3) *T. ceylonicus* Schiner
(4) *T. discrepans* Ricardo
(5) *T. flavissimus* Ricardo
(6) *T. flaviventris* Bigot
(7) *T. fuscicauda* Bigot
(8) *T. fuscicrura* Bigot
(9) *T. indiscriminatus* Ricardo
(10) *T. jucundus* Walker
(11) *T. puteus* Ricardo
(12) *T. rubidus* Wiedemann
(13) *T. speciosus* Ricardo
(14) *T. tenens* Walker

Of the above, the following nine species are exclusive to this region :—

(1) *T. angustilimbatus*
(2) *T. atrohirtus*
(3) *T. ceylonicus*
(4) *T. discrepans*
(5) *T. flavissimus*
(6) *T. fuscicauda*
(7) *T. fuscicrura*
(8) *T. indiscriminatus*
(9) *T. puteus*

Burma.—A long high range of hills divide Burma and India. The rivers Irawati, Salween and Sittang are perennial and flow towards the south and fall into the bay of Marttawan. The coastal areas have plenty of rainfall and are predominantly rice growing specially in the deltaic areas of the Irawati. Certain tracts are also rich in tropical forests of teak wood.

The genus *Tabanus* in this area is represented by nineteen species, namely :—

(1) *T. abscondens* Walker
(2) *T. birmanicus* Bigot
(3) *T. brunnipennis* Ricardo
(4) *T. flaviventris* Bigot
(5) *T. fulvimedius* Walker
(6) *T. fuscicauda* Bigot
(7) *T. fuscomaculatus* Ricardo
(8) *T. hirtistriatus* Ricardo
(9) *T. hybridus* Wiedemann
(10) *T. kakhyenensis* Senior-White
(11) *T. leucocnematus* Bigot
(12) *T. monotaeniatus* Bigot
(13) *T. orientis* Walker
(14) *T. oxyceratus* Bigot
(15) *T. rubicundus* Macquart
(16) *T. rubidus* Wiedemann
(17) *T. subcallosus* Ricardo
(18) *T. subcinerascens* Ricardo
(19) *T. tenens* Walker

Of these, the following four species are exclusive to the region :—

(1) *T. abscondens*
(2) *T. hirtistriatus*
(3) *T. kakhyenensis*
(4) *T. subcinerascens*

Perso-Baluchi-Afghanistan.—This region is separated from India by rocky mountain ranges, certain peaks of which are covered with snow. Most of the area is barren with sandy desert with very little vegetation. Rainfall is low, not exceeding seven or eight inches. In Afghanistan a few small rivers help in irrigating the surrounding plains whereas in Persia the areas near the Campean lake is fertile and cultivated. Baluchistan has extremes of climate and the land is infertile.

Six species of *Tabanus* are found in this area :—

(1) *T. adjacens* Ricardo
(2 *T. ditaeniatus* Macquart
(3) *T. eggeri* Schiner
(4 *T. glaber* Bigot
(5) *T. leleani* Austin
(6) *T. pulchellus* Loew

Of these four are specific to the region :—

(1) *T. adjacens*
(2) *T. eggeri*
(3) *T. glaber*
(4) *T. pulchellus*

The species *Tabanus nicobarensis* Schiner has been recorded from the Nicobar Islands only.

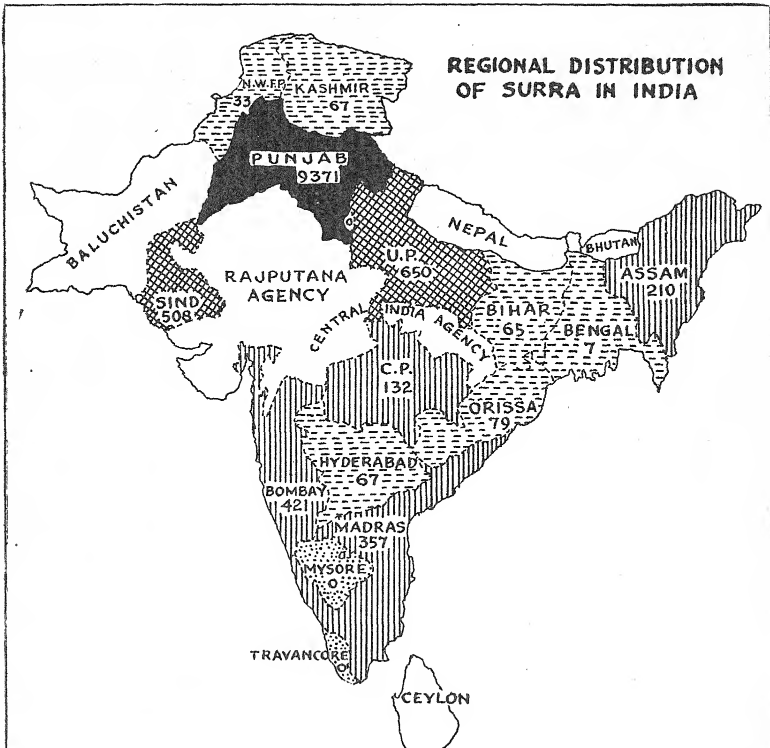
It may be remarked that of the seventyfive species of *Tabanus* recorded from India and the surrounding countries only seven have been incriminated in the transmission of surra. They are :—

(1) *T. ditaeniatus* Macquart
(2) *T. macer* Bigot
(3) *T. nemocallosus* Ricardo
(4) *T. rubidus* Wiedemann
(5) *T. striata*
(6) *T. tropicus* Panzer
(7) *T. virgo* Wiedemann

Of these, *Tabanus rubidus* Wiedemann has been incriminated as the most notorious carrier of surra. The evidence in respect of the other species as vectors of surra is meagre and in most cases confirmation is necessary. A thorough study of the bionomics of these species with special reference to their breeding and preferential feeding habits and their capacity for transmission of the disease is required.

The result of work done at the Indian Veterinary Research Institute on the vectors other than *Tabanus* involved in the transmission of surra, was reported by Basu [1947] at the Indian Science Congress.

The distribution of surra, the tabanid borne disease, in India must necessarily be co-extensive with that of its insect vector. Basu [1945] recorded the incidence of surra in the different States of the Indian sub-continent based on the political boundaries (*vide* Plate XXI and Table III).



Map showing number of cases (all species) reported during three years 1940–42. After Basu, B. C. [1945].

TABLE III

Surra cases (January 1940 to December 1942)

Province or State	Bovines	Equines	Camel etc.	Total
Assam	4	205	1	210
Bengal	0	7	0	7
Bihar	22	43	0	65
Orissa	21	58	0	79
Madras	265	92	0	357
Central Provinces	55	74	3	132
Bombay	50	371	0	421
Sind	37	404	67	508
Punjab	491	4,537	4,343	9,371
United Provinces	10	160	480	650
N. W. Frontier Province	0	33	0	33
Ajmer	..	..	..	..
Hyderabad	28	39	0	67
Kashmir	?	?	?	67
Travancore	0	0	0	0
Rampur	..	..	..	..
Patiala	..	..	..	..
Jaipur	..	..	..	..
Bikaner	..	..	..	..
Udaipur	..	..	..	..
Jind	..	..	..	..
Mysore	0	0	0	0
Baroda	21	13	0	34
Gwalior	0	26	19	45
Alwar	0	7	0	7
	1,004	6,069	4,913	12,058

N.B.—Reprinted from Indian J. Vet. Sci., XV, **4**, 278

In this paper efforts have been made to rearrange the incidence of surra on regional basis as has been done in case of tabanid flies. It is seen that in *Region 1* the incidence is varied. In Kashmir the incidence is reported to be moderately low, it is very high in portions of East Punjab and Himachal Pradesh and moderately high in certain portions of Uttar Pradesh and low in portions of Bihar, Bengal and Assam. No record of surra was available from Nepal, Sikkim and Bhutan. In *Region 2* the range of variation of incidence varies from moderately heavy to very low. No record was available from Vindhya Pradesh. In *Region 3* heaviest incidence was recorded in the portion of Punjab and moderately heavy infestation in Sind and part of Uttar Pradesh. The comparatively less infested areas of Kashmir, North West Frontier Province and Madhya Pradesh are also included in this region. No information was available from Greater Rajasthan, a camel raising area, and Baluchistan. In *Region 4* the incidence of surra in Bombay and Madras has been reported to be moderately high and Mysore has been reported to be free from it. No report of surra was available from Saurashtra, Kutch and Madhya Bharat. Incidence of surra in *Region 5* has been recorded very low.

However, with a view to establish more clearly the direct relationship between the disease and the transmitting vector, more detailed information will have to be collected to rearrange the distribution map of surra on regional basis.

Summary

To provide a ready, uptodate reference to the distribution of *Tabanus* flies in India, their incidence has been plotted in a map of India. For this purpose, the country has been divided into the following five regions as recommended by the Indian Council of Agricultural Research in connection with the orientation of different agricultural research organisations in India depending mostly on rainfall, soil, type of crop, etc.

Temperate Himalayan Region.—Nineteen species of *Tabanus* are recorded from this region, of which seven are specific to this region.

Eastern Wet Region.—Thirty-two species are recorded from this region of which nine are specific to this region.

North Western Dry Region.—Seventeen species are recorded from this region, of which two are specific to this region.

Southern Region.—Twenty species are recorded from this region, of which three are specific to this region.

South Coastal Wet Region.—This region is represented by ten species of which two are specific to this region.

The incidence of *Tabanus* flies in India has also been arranged according to its natural physical features, dividing the country into four regions, namely, Montane, Submontane, Pastural and Coastal. It is noted that as many as thirty species are recorded from the Montane region of which ten are specific. Submontane region has thirty-two species of which three are specific. Pastural region is represented by twenty-three species out of which seven are specific. Lastly, the coastal region is represented by fifteen species of which three are specific. *Tabanus rubidus* Wiedemann is found to be cosmopolitan in all the regions.

Out of the seventy-five species of *Tabanus* recorded from India and the surrounding countries, the following seven has been reported to be capable of transmitting surra: *Tabanus ditaeniatus* Mac., *Tabanus macer* Bigot, *Tabanus nemocallosus* Ricard., *Tabanus rubidus* Wied., *Tabanus striatus* Fabr., *Tabanus tropicus* Panzer and *Tabanus virgo* Wied.

The incidence of surra has been recorded to be the heaviest in the Punjab followed by Sind and Uttar Pradesh in its intensity. Comparatively lower incidence of infection has been observed in Assam, Madhya Pradesh, Bombay and Madras and the least infection has been noticed in Bengal, Bihar, Orissa, Hyderabad, Kashmir and North West Frontier Province. Mysore has been reported to be free from the disease.

ACKNOWLEDGMENT

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APPENDIX

Species of Tabanus recorded from India and surrounding territories

Species	*Place recorded from*
1. *T. abscondens* Walker	Burma, N. Chin Hills, Dawnat Range (Tenasserim), China
2. *T. adjacens* Ricardo	Perso-Baluchistan Frontier
3. *T. albocostatus* Bigot	India
4. *T. albofasciatus* Ricardo	Shillong
5. *T. angustilimbatus* Senior-White	Marble Valley (Ceylon)
6. *T. atrohirtus* Ricardo	Ceylon, Sumatra (Mount)
7. *T. auriflamma* Walker	Sylhet, Sibsagar (Assam), Naga Hills, Cachar
8. *T. auristriatus* Ricardo	Gersoppa (N. Kanara), Coonoor
9. *T. bicinctus* Ricardo	N. Kanara, Mohand (U. P.), Coonoor, Pollibett (S. Coorg), Sampaji Ghat (Coorg), F.M.S., Formosa
10. *T. birmanicus* Bigot	Burma, Lushai Hills, Nongpoh (Khasia), F.M.S., Formosa
11. *T. brunnipennis* Ricardo	Basi (N. Kanara), Gorakhpur (U. P.), Pusa, S. Malabar, Mohnyin (U. Burma), Bistupur (Bankura Dist.), Puri, Dinapur (Coorg), Khurda, Santikoppa (N. Coorg), Polibetta (S. Coorg), Ranchi, Samastipur (Bihar), Kasaragode (S. Kanara), Siam
12. *T. ceylonicus* Schiner	Ceylon, Trincomali (Ceylon), Malay Penin., Java, Sumatra
13. *T. conicus* Bigot	India, Calcutta, Java, Formosa, F.M.S.,
14. *T. consanguineus* Macquart	Malabar Coast
15. *T. demellonis* Senior-White	Combarjua (Port. India)
16. *T. discrepans* Ricardo	Ceylon
17. *T. ditaeniatus* Macquart	N. India, Jhelum Dist., Perso-Baluchistan Frontier, Pusa, Bengal, Madras Coast, Ambala, Balighai (Orissa), Port. India, Mohand (U. P.), Tuticorin, Pathankot (Crosse), D. Gazipur (Crosse), Dera Ismail Khan, Mianwali (Punjab), Seistan, Tank (N. W. F.), Coimbatore (S. India), Japan, China, Hongkong, Africa (throughout), Mauritius, Reunion

18. *T. diversifrons* Ricardo	Shillong, Khasia Lower Ranges, Buxar, Duars, Sylhet, Darjeeling Dist., Calcutta
19. *T. eggeri* Schiner	Pers. Seistan, South Europe, Asia Minor, Egypt, Palestine
20. *T. excelsus* Ricardo	Mashobra, Mohand (U. P.), Dehra Dun, Kangra Dist
21. *T. explicatus* Walker	Sikkim, Khasia Hills, Coonoor
22. *T. flavicinctus* Ricardo	Khasia Hills, Nilgiris, N. Kanara, Mohand (U. P.)
23. *T. flavissimus* Ricardo	Java, Pundaluoya (Ceylon)
24. *T. flaviventris* Bigot	Sibsagar (Assam), Ceylon, Calcutta, Tenasserim, Bihar
25. *T. fulvimedius* Walker	Nepal, N. Chin Hills, India, Formosa
26. *T. fuscicauda* Bigot	Ceylon, Pundaluoya (Ceylon), Shewgu (Burma), Perademiya (Ceylon), Andaman Islands
27. *T. fuscicrura* Bigot	Ceylon
28. *T. fuscomaculatus* Ricardo	Myitkina Dist. (U. Burma), Sikkim, Masuri, Kumaon, Manipur
29. *T. glabaer* Bigot	Helmand River (Afghanistan), Kashgar, Mesopotamia, Persian Seistan
30. T, hirtipalpis Ricardo	Bichiakoh (Nepal)
31. *T. hirtistriatus* Ricardo	Dawnat Range (Tenasserim), F.M.S.
32. *T. hirtus* Walker	India, Phagu (Simla D.), Nainital D., United Provinces, Mukteswar (Kumaon)
33. *T. hybridus* Wiedemann	Burma, Sylhet, F.M.S., Borneo, S. China
34. *T. indianus* Ricardo	Kadra (N. Kanara), Goa (Port. India), Santikoppa (Coorg), Polibetta (Coorg), Makut (Coorg,) Mercara (Coorg) Poona, Foochow (China), Formosa, Hongkong
35. *T. indiscriminatus* Ricardo	Ceylon
36. T. joidus Bigot	Sibsagar (Assam)
37. *T. jucundus* Walker	Pundaluoya (Ceylon), Kohat, N.W.F. Masuri, Igatpuri (W. Ghats), Mohand (U. P.), Hongkong
38. *T. kakhyenensis* Senior-White	Mohnyin, N. Toungoo, Phynmana (all U. Burma)

39. *T. Khasiensis* Ricardo	Khasia Hills, Meerut, Sinabang (Siam), Penang
40. *T. leleani* Austin	Kangra Valley, Seistan, Quetta, Attock, Near Srinagar (Kashmir), Algeria to Mesopotamia
41. *T. leucocnematus* Bigot	India, Lushai Hills, Khasia Hills Coonoor, Katha (U. Burma), Darjeeling.
42. *T. leucohirtus* Ricardo	Kanara (Bombay)
43. *T. leucopogon* Bigot	Sikkim
44. *T. macer* Bigot	India, Jhelum Dist., Pusa, Goa (Port India), Mohand (U. P.), Trichinopoly Pathankot, Murree, Kathgodam, Dojaion Rawalpindi, Chamba State (Punjab), Nagpur, Dharwar, Coimbatore Taliparamba (N. Malabar), In train between Rameswaram and Madras
45. *T. manipurensis* Bicords	Manipur
46. *T. melanognathus* Bigot	Purneah (N. Bengal), Laos (Siam)
47. *T. monotaeniatus* Bigot	India, N. Khasia Hills, Nongpoh (Khasia), Sibsagar (Assam), Yunnan Mohnyin (U. Burma)
48. *T. nemocallosus* Ricardo	Pusa, Jhelum District
49. *T. nephodes* Bigot	Naga Hills, Sibsagar (Assam)
50. *T. nicobarensis* Schiner	Nicobar Islands
51. *T. nigropictus* Macquart	India
52. *T. obconicus* Walker	Central India, Bombay, Belgatchia (Bengal)
53. *T. optatus* Walker	Belgatchia (Bengal), Bongaon (Bhagalpur Dist.), F.M.S., Sumatra, Borneo
54. *T. orientis* Walker	Nepal, United Provinces, Nainital, Thandiani (Himalayas), W. Bhutan, Masuri, Dongagalli (Murree Yunnan), Muktesar, Mohand, Sikhim Jhelum Dist., Kangra Dist., Dalhousi
55. *T. oxyceratus* Bigot	India, Cheena Forest (Himalayas), N. Chin Hills, Masuri
56. *T. provincialis* Ricardo	Arian Kava (Travancore), Kurseong
57. *T. pulchellus* Loew	Seistan, Mesopotamia, Asia Minor, N. Africa, Cyprus, Perso-Afghan Frontier
58. *T. puteus* Ricardo	Trincomali Dist., Colombo, Habarane, N.C.P. (all Ceylon)

59. *T. rubicundus* Macquart	India, Chargola Valley (Sylhet), Sylhet, Khasia Hills, Petsut, Katha (U. Burma), Sibsagar (Assam), Java
60. *T. rubidus* Wiedemann	Bengal, Moulmein, Bombay, Pusa, Sibsagar, Khasia Hills, Partabghar (U.P.), Nepal, East India, Trinco. Dist. (Ceylon), Ambala, Calcutta, Muktesar, Matheran (W. Ghats), Goa (Port India), Jhelum Dist., Masuri, Dharamsala (Crosse), Kangra (Crosse), D. Ghazipore (Crosse), Hopin (U. Burma), Chapra (Bihar), Buxar (Bihar), Coimbatore, Rawalpindi, Taru near Peshwar, Giridhi, Khumbala, Khurda, Khasi Hills, Bareilly, Midnapur, Kashmir, Nongpoh (Assam) Manbhum, Delhi, Cuttack, Dera Ismail Khan, Dumraon (Bihar), Kasargode (S. Kanara), Asia, Java, Sumatra, F.M.S. Siam, Hongkong, Cochin China, Annam
61. *T. rufiventris* Fabricius	E. India, Assam, Karwar (N. Kanara) Pusa, Lr. Khasia Hill, Lushai Hills, Sumatra, F.M.S., Hongkong, Formosa
62. *T. sexcinctus* Ricardo	Lushai Hills, Masuri, Formosa.
63. *T. speciosus* Ricardo	Travancore, Trinco. Dist. (Ceylon) Madras, Hazaribagh (Bihar), Angul, Bombay, Cuddapah (Madras Pres.), Chapra, Khurda, Santikoppa (N. Coorg), Coimbatore, Dum-raon, Buxar, Bhagalpur
64. *T. striatus* Fabricius	Kohat, Jhelum D., E. India, Pusa, Punjab, Bombay, Hissar (Punjab), Belgatchia (Bengal), Midnapur, Angul, Monghyr, Rawalpindi, Lahore, Bhagalpur, Suri, Cuttack, Daltonganj (Bihar), Darjeeling, Burdwan, purulia, Ludhiana (Punjab), Kathgodam, Coimbatore
65. *T. subcallosus* Ricardo	Masuri, Dalhousi, Muktesar, Dogaon, Meerut, Muzaffarnagar, Dera Dun (U. P.), Pokokku (Burma), Dunera Hills (Gurdaspur D)., Kangra (Punjab), Amparad (near Mukteswar)

66. *T. subcinerascens* Ricardo	N. Chin Hills
67. *T. subhirtus* Ricardo	Bengal, Bombay, Java
68. *T. sufis* Jaennicke	Punjab, N. Africa, E. Africa
69. *T. tenbrosus* Walker	Kanara
70. *T. tenens* Walker	E. India, Ceylon-general, Bassein (Bombay), Madras, Pusa, Bengal-general, Burmah, Gonda D. (U. P.), E. Coast, Sylhet, Manipur, Mohmand Pass (N. W. F. P.) S. India-general, Goa (Port. India), Dehra Dun, Kasargoda (S. Kanara), Coimbatore, Berhampur, Java, Sumatra, F.M.S., Phillippines, China
71. *T. trinominatus* Senior-White	India
72. *T. tropicus* auoct.	Ind. *nec* Panzer (1794) Kathgodam, Mukteswar (both Himalaya), Padda Sultan (Helmand), Java
73. *T. tuberculatus* Ricardo	Companiganj (Sylhet), Belgatchia (Bengal), Pusa, Villupuram (S. Arcot), Stehands (Kistna)
74. *T. virgo* Wiedemann	India, Madras, Mysore, Calcutta, Pusa, Mohand (U. P.), Jhelum Dist., Vizianagram (S. W.), D. Ghazipore (Crosse), Kangra (Crosse), Kotla (Crosse), Chapra (Bihar), Chicacole Road (Madras Pres.), Suri (Bihar), Sohawa (Punjab), Hazaribagh, Rawalpindi, Khurda, Cuttack, Bareilly, Purulia (Bihar), Angul, Bowrah (Champaran)
75. *T. wyvillei* Ricardo	Narkunda, Kasauli (both W. Himalayas)

BIKANER WOOL, A LEADING CARPET WOOL, AND ITS IMPROVEMENT*

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As a result of the increasing strain to keep up with the world economy and to raise the standards of living, serious efforts have been made to utilize the internal resources in most Asiatic countries. The improvement in agricultural economy has thus become of vital importnace; this includes the livestock industry, which forms the backbone of agricultural countries like Inida and China. Closely associated with the larger class of livestock is sheep, the importance of which has been recognized since the earliest of times as a source of food and clothing.

The need for improving sheep and wool in India has attracted special attention in recent years and every effort is being made for the development of the industry. The average production of wool per sheep is reported as 1·9 lbs. a year [3], which is probably lower than that for any other sheep-raising country; the entire yearly production of 54·33 million lbs. [5] from a quality standpoint is classed as a coarse carpet wool, suitable only for the production of carpets and rough blankets. In spite of producing surplus wool, with an export figure of about 41·14 million lbs. [5] every year, the country has to import 19·24 million lbs. of apparel wools in order to meet its needs for the manufacture of clothing. The total export goes into the international trade as carpet wool. Besides the need for increasing the quantity of wool per sheep, it seems that improvement of quality, both for the production of apparel wool to meet the domestic consumption and for the development of ideal carpet wool for export, would be a befitting plan for any progressive program, with due regard to environmental and feeding conditions.

From a preliminary survey by the Marketing Department of India [3], it appears that although the foundation stock is inferior, potentialities for improvement in certain breeds are not entirely lacking. One of the promising breeds of sheep, of country-wide importance, is the Bikaner, native to the northwestern parts of India in the Rajasthan Union. It derives its name from its homeland, the Bikaner State. The breed is reported to produce one of the finest carpet wools in the world [16], and the average fleece weight is reported to be as high as 6·5 lbs. [23]. Due to the superior quality of the sheep and its wool, the breed has found favour in many other states of the country—Madras, United Provinces, Bihar, Orissa, and Assam—to replace the native inferior types or for crossbreeding. In

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spite of varied climates, which in many cases are too humid, with high rainfalls, as compared with its arid home-region, the breed is reported flourishing in Assam [15], Orissa [27], and Madras [6]. The breed already yields an enormous influence in Rajputana, Punjab, and United Provinces, and, with the growing interest to introduce the breed in southern and central India, Bikaner sheep and wool have become important in all regions of India. With a view to explore the possibilities and the potentialities for improvement of this sheep and wool, 8 representative commercial samples were analyzed in the Wool Department, University of Wyoming, Laramie, Wyoming.

Position of Bikaner Wool

Bikaner wool is unique for its length, color, quality, and loftiness as a product best suited for carpets. It always commands the highest prices in the local markets as compared with other wools, which usually are double in amount by weight [4]. Because of its superior quality, the wool is mixed by the traders with inferior wools from adjoining areas and exported to foreign countries under the trade name of Vicanere wool [4]. In recent years, wool from certain localities has been so highly appreciated for its fineness and quality that certain authorities have been inclined to believe that the wool is more suitable for clothing purposes than for carpet manufacture.

A part of the wool produced is utilized by farmers in hand spinning and weaving for making articles of native dress and blankets, while a certain percentage goes into the manufacture of carpets and rugs. A major portion is exported from the home-region to markets in other parts of India for use in the mills and for foreign trade. The 3 types of wool—Magra, Chokla, and Nali—classified according to the area of production, fineness, and quality, are all mixed together before export. The wool that goes to the mills is utilized for making carpets, blankets, and woolen materials. The lofty character of the wool makes it fairly suitable for the manufacture of hosiery and blankets. Better sorts are sometimes used by the mills for tweeds.

Materials and Methods

Origin of the Wool Samples

The wool samples analyzed in this study were obtained through the courtesy of Mr. B. N. Handa, Superintendent, Government Livestock Farm, Hissar, India. In order that the samples should represent purity of the type, these were originally obtained from the Bikaner State from a dealer in the Sujanghar area who is well known for handling good quality of wool. A total of 28 samples were received. Only 8 samples representing extreme and average types in fineness and other qualities were picked by visual examination for study.

preparation of Samples

Each sample was divided into 8 approximately equal subsamples. Great care was taken to prevent breaking of the fibers. By taking small portions from each subsample, several duplicate composite samples could be used for the study of different characteristics.

Before analysis the representative samples were scoured in order to remove extraneous matter. The method of scouring suggested by Johnston [22] was employed. Before analysis a final rinse in carbon tetrachloride was given. All burrs or other vegetable matter were carefully picked out from each sample by hand; the sample was then blended, by repeated splitting of the staples, for the study of different characteristics.

Techniques used for Analysis

The various tests and determinations made in the analysis of the samples included: medullation test; stretched fiber length measurements: fiber thickness; contour ratio; and surface scale structure for fiber types.

Bikaner wool is of mixed type and contains different types of fibers—namely, wool, heterotype, hair, colored fibers, and kemp. Except for the colored fibers, the distinction between the fiber types is made according to the degree of medullation—no medullation (wool), partial medullation (heterotype), or complete medullation (hair). The kemp fiber is a characteristic "dead" fiber, and is comparatively thick, short, straight, and in most cases opaque white like burnt bone.

Various workers, such as Blyth [9], Roberts [29], Wilson [33], Lochner [24], Elphick [18], Darling [17], Bulgaru [12], Munz [25], and Burns, Johnston, and Chen [14], conducted analyses of mixed wools; their basic procedure was to determine the proportion of different types of fibers, although using slightly different names in some instances. This is important because all the fibers possess variable physical properties, and a change in their proportion is likley to alter the character and nature of a wool. The method for the analysis of mixed wools has now been adopted universally. Although the analysis of fiber types is a useful guide in evaluating the quality of mixed wools from a manufacturing viewpoint, it does not give the actual amount of medullation in a sample due to varying amounts of medullated and non-medullated portions in the heterotypes. This estimation is essential for a critical selection of sheep in any breeding-improvement program. Accordingly, the medullation test was carried out under two different techniques—fiber-count analysis, and cross-sectional medullation test.

For a counting analysis, duplicate composite samples were separated into the different types of fibers by using Elphick's benzol test. However, it was found necessary for efficient results that benzol, with a refractive index of 1·50, be employed. In case of doubt, a microscope was employed for the identification of the fibres. A white paper background was used to identify colored fibers. Fibers in each class were counted and their proportions worked out mathematically.

For the cross-sectional medullation test, duplicate composite samples were used for each type of wool. Hardy's technique [19] was used in making cross sections. Three sections were made of each sample—namely, one at the base, one at the middle, and one at the tip. These sections were projected, using 500× magnification, on a white surface of 25 cm. ×10 cm. area, and the number of medullated and non-medullated fibers were counted and recorded. The total number of medullated and non-medullated fibers in the different samples were determined. From these figures the percentages of the two fiber types were calculated.

Stretched fiber length measurements were made of the fiber types which were separated out in the count analysis. Burns' improved technique [13] was used for the measurements. One hundred fibers of each type were measured, except when their total number in the sample was smaller.

Thickness measurements of each sample as a unit and of the fiber types after separation were made separately. Duplicate composite samples were used in the former case, while the fiber types that were used for the stretched fiber length measurements were utilized in the latter case. The longitudinal short-section method was used in the thickness measurements [1]. The minimum number of readings to come within a prescribed limit of error was first worked out by the formula employed by Roberts [28], and all measurements were made accordingly. Only 100 readings were recorded to measure the fiber types.

The contour ratio, which expresses the ellipticity of the fiber cross section, was measured in terms of the ratio of the major to the minor axis [31]. Barker [8] has proved that in two wools of the same fineness, the more circular wool spins better. Hardy's technique [19] was used in making the cross sections. The cross-sectional fiber images were projected and photographed on sensitized bromide paper.

The major and minor axes of each fiber cross section were measured with the bi-diameter scale and recorded. The contour ratio was worked out from these readings.

The study of scale structure was made by obtaining surface impressions with the aid of a thermoplastic film according to the technique developed by Hardy and Plitt [20]. Until recent years, an efficient method for the study of scale structure had been a difficult problem, but the above method gives satisfactory results. The impressions on the film were mounted on a glass slide and projected at a magnification of 500 diameters. Photographs of projected images were then obtained on sensitized paper, showing representative fibers for the different measurements.

The attributes studied were the scale outline, the number of scales per unit length, the average visible scale height, the number of scales along the margin per 100μ length, and the relationship of scale height to fiber diameter.

Results and Discussion

Medullation Test

The results of modullation test both by fiber type analysis and cross-sectional technique are presented in Table I. The fiber-count analysis reveals that the average fiber type content in the 8 samples is 58·6 per cent true wool, 29·8 per cent heterotype, 11·4 per cent hair, and 0·1 per cent kemp. The largest variation is in the true wool fibers—from 16·8 per cent to 96·5 per cent; variation in the heterotypical fibers is from 3·5 per cent to 49·3 per cent, and in hair from 11·3 per cent to 33·6 per cent. Four samples show total freedom from hair and 6 samples are free from kemp. The average content of kemp fiber is only 0·1 per cent, which may be considered negligible. No colored fibers were found in any of the 8 samples.

By the cross-sectional image method, the average percentage of non-medullated fibers is 70·7 per cent, and of medullated fibers 29·3 per cent. The range of

variation is from 23·8 per cent to 98·9 per cent in the former case, and from 1·1 per cent to 76·2 per cent in the latter.

TABLE I

Results of Medullation Test

Sample number	Percentage of fiber types by count				Percentage of total fibers by cross—section image	
	True wool	Heterotype	Hair	Kemp	Non-medullated	Medullated
1	57·8	41·9	—	0·3	91·4	8·6
2	39·4	49·3	11·3	—	54·1	45·9
3	38·9	39·9	21·2	—	51·4	48·6
4	96·5	3·5	—	—	98·9	1·1
5	16·8	49·1	33·6	0·5	23·8	76·2
6	94·6	5·4	—	—	98·1	1·9
7	80·7	19·3	—	—	95·8	4·2
8	44·6	30·1	25·3	—	52·4	47·6
Average	58·6	29·8	11·4	0·1	70·7	29·3

From both of the above tests it is evident that there is a wide range of variation in the true wool fibers of the samples. In samples Nos. 4 and 6 the true wool content approaches very near to total freedom from medullation. According to various workers, such as Bryant [11], Barker [8], Elphick [18], Waters [32], and Nanda, Singh, and Mogre [26], medullation in wool is probably inherited. The results thus show high potentialities for total elimination of medullation in the breed and for the evolution of a true wool type of sheep by selective breeding. Townend and McMahon [30], by subjecting large quantities of Romney wool to various stages of manufacture, noticed that hairiness up to the extent of 6 per cent, as determined by McMahon's Medullometer, did not affect the processing properties of the wool and produced no marked difference in the appearance and handle of either woven or knitted fabrics. The hairiness could not be detected in dyed cloths. An extremely low medullation content in samples Nos. 4, 6, and 7 shows that suitable sorts can be picked up from the Bikaner wool which are suitable for the manufacture of clothing material.

Stretched Fiber Length Measurements

The stretched length measurements of the different fiber types and the percentages of variation are shown in Table II.

The range of average length in the true wool fibers is from 6·25 cm. to 14·33 cm., with a coefficient of variability range of from 7·8 per cent to 26·2 per cent. The

average length of 800 fibers in all samples was 10·38 cm., with a coefficient of variability of 31·0 per cent.

TABLE II

Stretched Fiber Length Measurements of Fiber Types

Sample number	True wool		Heterotype		Hair	
	Mean (cm.)	Coefficient of Variability (%)	Mean (cm.)	Coefficient of variability (%)	Mean (cm.)	Coefficient of variability (%)
1	10·24	12·2	18·17	14·7	—	—
2	9·07	10·7	13·47	12·6	10·16	7·8
3	8·28	14·6	14·47	11·2	—	—
4	14·33	18·0	17·58	17·3	15·21	10·5
5	6·25	15·4	9·81	16·1	13·31	12·9
6	13·87	26·2	17·08	15·9	—	—
7	10·39	18·3	13·60	12·1	—	—
8	10·57	7·8	13·05	14·3	14·77	10·1

The range of average length in the heterotypical fibers is from 9·81 cm. to 18·1 cm., with a coefficient of variability range of from 11·2 per cent to 16·1 per cent. The average length of 714 fibers in all samples was 14·34 cm., with a coefficient of variability of 23·1 per cent.

The range of average length in the 4 samples for hair fibers is from 10·16 cm. to 15·21 cm., with a coefficient of variability range of from 7·8 per cent to 12·9 per cent. The average length of 400 fibers in all samples as a whole was 13·21 cm., with a coefficient of variability of 20·7 per cent.

Although there is good uniformity in the fiber types of individual samples, the variability is larger if the samples are taken as a whole. The highest variability is noticeable in the true wool fibers, and the lowest in the hair fibers. If the hair and heterotypical fibers were eliminated through selective breeding, there would be ample scope to improve the length of the wool.

Fiber Thickness Measurements

The thickness measurements of the entire samples and of the fiber types, with the variabilities, are given in Table III.

The average range in the thickness measurements of 8 samples is from 26·55μ to 47·23μ, with a coefficient of variability range of from 32·1 per cent to 59·2 per cent. The average fiber diameter of 8 samples as a whole is 34·68μ. The minimum measurement was 12·5μ for a true wool fiber, and the maximum was 117·5μ for a hair fiber.

The range of average thickness for the true wool fibers in the 8 samples is from 16·45μ to 31·00μ, with a coefficient of variability range of from 3·8 per cent to 35·9 per cent. The average thickness of 800 fibers in all samples was 24·16μ with a coefficient of variability of 29·9 per cent.

TABLE III

Thickness Measurements of Entire Samples and of the Fiber Types

Sample number	Entire sample		True wool		Heterotype		Hair	
	Mean (μ)	Coefficient of variability (%)	Mean (μ)	Coefficient of variability (%)	Mean (μ)	Coefficient of variability (%)	Mean (μ)	Coefficient of variability (%)
1	26·77	49·2	16·45	18·6	33·80	29·9	—	—
2	34·80	43·3	23·83	16·8	32·65	19·9	55·45	29·5
3	34·66	36·2	22·63	12·9	31·60	20·3	—	—
4	27·56	32·1	25·52	30·5	49·62	36·8	45·65	15·3
5	46·67	59·2	19·85	3·8	30·80	24·8	71·62	25·5
6	26·55	34·2	25·70	35·9	44·80	18·7	—	—
7	33·18	32·2	28·12	25·6	49·10	14·3	—	—
8	47·23	42·8	31·00	17·8	40·45	20·8	17·68	25·2

The range of average thickness for heterotypical fibers in all samples is from 30·80μ to 49·62μ, with a coefficient of variability range of from 14·3 per cent to 36·8 per cent. The average thickness of 800 fibers in the 8 samples as a whole was 37·74μ, with a coefficient of variation of 26·8 per cent.

The range of average thickness for hair fibers in the 4 samples is from 45·65μ to 71·62μ, with a coefficient of variation range of from 15·3 per cent to 29·5 per cent. The average thickness of 400 fibers in the 4 samples was 63·56μ, with a coefficient of variation of 33·4 per cent.

The thickness measurements show a high variation both in the entire samples measured individually and in the fiber types. The finest samples, with thicknesses of 26·55μ and 26·77μ, show variations of 34·2 per cent and 49·2 per cent. The greatest uniformity is evident in the true wool fibers, whereas the hair fibers are the most variable. Barker [7], in his report on the Shantung and Woozie sheep of China, does not favor a variation higher than 25 per cent in wools to be used for clothing material. In the present study the variation is higher than 25 per cent both in the entire samples and in the fiber types. For an intended improvement of wool as a whole, it appears that the greatest emphasis should be on attaining uniformity of fineness of fibers. The wide range of variation in the true wool of from 3·8 per cent to 35·9 per cent indicates high potentialities to achieve this objective.

The amount of medullation and the thickness measurements of different samples apparently show a direct relation to one another. The thickness increases with increase of medullation in the sample. But, even with such increases, hair fibers with a thickness of 30μ and wool fibers with a thickness of 50μ are also encountered, which evidently shows that thickness alone does not control medullation in wool; some other factor, probably genetic, is partly, if not entirely, responsible.

Contour-Ratio Measurements

The contour-ratio measurements in the 8 samples are shown in Table IV.

The average contour ratio in the 8 samples is 1·284, with a range of from 1·170 to 1·382. According to trade opinion, the spinning properties of wool can be divided into 3 groups [31]—*i.e.*, very good spinning, with a contour ratio of below 1·2; average spinning, with a contour ratio of from 1·2 to 1·22; and fair spinning, with a contour ratio above 1·22. The results thus show that although considered as a whole, the wool samples show fair spinning property—1 of the samples possesses very good, 1 medium, and 6 fair spinnability. The potentialities for a very good spinning property, however, are evident.

The contour ratio seems to be related to the percentage medullation in the different samples. With increase of medullation there is an increase in contour ratio and a poorer spinning property. This is further proof to the general assumption that increase in medullation lowers the spinning property of wool.

TABLE IV

Contour-Ratio Measurements in the Different Samples

Sample number	Average contour ratio	No. of fibers measured
1	1·282	104
2	1·300	101
3	1·355	100
4	1·226	130
5	1·373	120
6	1·170	160
7	1·256	130
8	1·382	180

Surface Scale Structure on Fiber Types

The various characteristics of the scale structure of the fiber types in the different samples are shown in Table V.

The average number of scales on true wool fibers per in. length is 1,900, with a range of from 1,700 to 2,300 ; and on heterotypical and hair fibers is 2,788, with a range of from 1,800 to 3,400. When calculated on the basis of 100μ width and the same length, the average number is 10,797, with a range of from 9,000 to 13,571, in the former case ; and 5,332, with a range of from 3,214 to 7,750, in the latter. The average scale height is 16·7μ on the true wool fibers and 26·4μ on heterotypical and hair fibers.

Although the number of scales per unit length on the heterotypical and hair fibers seems larger than that on the true wool fibers, it is half as much when compared on the equal basis of the thickness of the fiber.

The number of scales along the margin per 100μ length of fiber was also worked out in the fiber types. The average for the true wool fibers was 6·4, with a range of from 5½ to 8 ; that for the heterotype and hair fibers was 5, with a range of from 4 to 7.

A relationship of scale height to fiber thickness, designated as the " Scale Index " by Hausman [21], was also calculated for fiber types in individual samples.

TABLE V

Results of the surface scale structure of fiber types

Sample number	No. of scales per in. length		No. of scales per in. length and 100μ width		Average scale height (μ)	
	True wool	Hair or heterotype	True wool	Hair or heterotype	True wool	Hair or heterotype
1	2,300	2,400*	11,250	6,000*	17·2	36·6*
2	2,000	3,400†	12,500	4,473†	16·8	17·0†
3	1,900	2,200†	10,611	6,111†	16·8	36·4†
4	1,800	1,800*	9,000	3,214*	16·0	22·4*
5	1,800	4,200†	10,000	5,250†	18·0	25·6†
6	1,900	2,000*	13,571	3,000*	13·2	24·4*
7	1,800	3,000*	10,000	7,750*	18·0	29·0*
8	1,700	3,200†	9,444	4,848†	17·6	20·0†
Average	1,900	2,788	10,797	5,332	16·7	26·4

* Heterotypical fibers.

† Hair fibers.

The average figure for the fiber types was 0·972 for wool, 0·687 for heterotypes, and 0·464 for hair fibers. It appears that as the diameter of the fiber increases there is a decrease in the " Scale Index".

The shape of the scales on the true wool fibers was of annular or coronal form (single scale along the width of the fiber), as described by Blyth [10], and resembled the segments of a young bamboo cane. In the case of heterotypical and hair fibers, the shape of the scales was polygonal or flattened. Various forms of pentagonal and hexagonal shapes were seen. It was apparent that with a smaller thickness the shape of the scales was more or less of a columnar type, but it inclined to become rectangular and flattened with increase in the thickness of the fiber.

Summary

Eight samples illustrating average and extreme types of Bikaner wool were selected from 28 samples received from the Government Livestock Farm at Hissar, India. These 8 samples were analyzed for fiber types, dividing them into true wool, hair, heterotype, and kemp fibers. Both the benzol test and microscopic cross-sectional images were used in the classification. Fiber thickness, stretched fiber length, and contour ratio were determined for the 8 samples along with the surface scale measurements for the different fiber types.

Conclusions

The following conclusions are evident from the results obtained from this study:

1. The results of the count analysis of fiber types and their dimensional characteristics in the 8 samples as a whole show that the wool is very near to the carpet wool standards as worked out by Burns, Johnston, and Chen [14] and the Southwestern Sheep Breeding Laboratory at Fort Wingate, New Mexico, from their work on Navajo Sheep [2]. However, it appears that the variation in the length and fineness of fiber types is slightly larger—by 4 per cent to 5 per cent. The average thickness of the heterotypical fiber is 7μ greater than desirable. The Bikaner wool as a commercial type can therefore be considered as very good for carpet and rug manufacture, although some improvement in attaining uniformity of fiber thickness is necessary in order to fix it as an ideal carpet type of wool. Individual samples Nos. 4 and 6, with high true wool contents of 96·5 per cent and 94·6 per cent, respectively, seem too good for ideal carpet wool; Nos. 5 and 8 with true wool contents of 16·8 per cent and 44·6 per cent, respectively, appear rather poor in this respect. Samples Nos. 1 and 7 are very close to the required standard, and Nos. 2 and 3 are slightly poorer for this purpose.

2. If the average proportion of fiber types is considered to be very near to ideal carpet wool by count analysis, the cross-section medullation test of the same samples reveals that by this method an ideal carpet wool should show 75 per cent non-medullated fibers and should not exceed a limit of 80 per cent.

3. Although the average quality of the samples reveals that the wool in a mixed form is best suited for carpets, the extremely low amounts of medullation in certain individual samples show that selected sorts can be utilized for the manufacture of clothing material. The wide range of medullated fibers in the different samples indicate further that there are high potentialities for evolving an apparel-wool type of sheep by selective breeding. It is highly desirable to eliminate kemp entirely from the fleece.

4. The analysis of medullation in the individual samples indicates that there are great possibilities for improving the wool for apparel fabrics, the variation in the fiber thickness being rather large to require particular attention. Even the finest samples, with average thicknesses of 26·55μ and 26·77μ, show variations of 34·2 per cent and 49·2 per cent. For any program of improvement, therefore, the greatest emphasis should be to attain the uniformity of fibers. The uniformity in the case of true wool fibers is fairly satisfactory.

5. The stretched fiber length of different types of fibers in the individual samples do not show any marked elongation of the outer coat compared to the inner coat as seen in most of the poor carpet wools. This characteristic is highly desirable for the spinning value of carpet wools.

6. There appears to be a relationship between the amount of medullation and the thickness of the entire sample, the diameter increasing with increased medullation in the sample. However, hair fibers with a diameter of 30μ and wool fibers with a diameter of 50μ show that the diameter alone does not control medullation in wool, and some other factor, probably genetic, is partly, if not entirely, responsible.

7. The average contour ratio of the 8 samples is 1·284, designating a fair spinnability according to the trade opinion. One of the samples showed a very good spinnability, with a contour of 1·179, and one showed a medium spinnability. This again reflects possibilities for improvement. Higher contour ratio seems to be related to higher amount of medullation in the individual samples.

8. The average number of scales on true wool fibers with an average thickness of 17·2μ is 1,900 along one surface, which is very close to that for the fine wool Merino. When compared on the equal basis of unit length and 100μ width, the number of scales on the true wool fibers was double that found on the heterotypical or hair fibers. The outline of the scales is uniform, regular, and annular on the true wool fiber, but is irregular, with pentagonal, hexagonal and rectangular forms, on the hair and heterotypical fibers.

9. For a mass improvement of Bikaner sheep it seems necessary that only tested rams be used for breeding which show a desirable amount of medullation in the fleece and possess maximum uniformity in fiber thickness.

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TYPES AND DIMENSIONS OF FIBER SCALES FROM THE WOOL TYPES OF DOMESTIC SHEEP AND WILD LIFE*

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THE study of the surface structure of wool and hair fibers is an important aid in the identification of fiber types. Besides affording a knowledge of fiber types, such a study would help determine the " healthy " state of fibers with respect to the number of scales per unit length and their shape and dimensions. The visible scale height seems to be an important characteristic for differentiation between wool and related hair fibers such as mohair and camel's hair. A special study of the epidermal structure is sometimes necessary in blends of wool and related fibers which can only be distinguished by the difference in the scale formation.

Apart from ascertaining the number of scales per unit length of the fiber and their shape in relation to the felting properties of wool, some workers have tried to relate these characteristics to the different species or breeds of sheep. A study of the surface scales—the form and surface dimensions of the scales—was made on fibers from a number of different wool samples representing different breeds and countries, including some wild sheep. The study included determinations of the number of scales per unit length of the fiber, the scale formation or outline, and the scale height, with some dimensional measurements ; the object was to ascertain whether these characteristics were : (*a*) related to different species or breeds of sheep ; (*b*) specific for different wool types; (*c*) specific enough to enable identification of fiber types in mixed wools.

All classes of wools, such as fine, medium, long, crossbred, and carpet types were represented in the study ; in the case of mixed wools, the different types of fibers, such as true wool, hair, and hemp, were examined separately.

Classification of Scale Structure

Various workers have tried to classify scale structure by a description of their general outline. Blyth [1] described two types of scale structure on the wool fibers—the wool type and the reticulate type. The former in its simplest form is of coronal pattern, each scale encircling the shaft of fiber and resting in the cup formed by the scale beneath it. In very fine fibers, the ectal edge of the scales is convexly curved, is faintly and irregularly serrate, and points distally. In thicker fibers, more than one scale to the circumference can be seen ; these tend to be broad and shallow,

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and are either convexly curved or their free edges trace out a wave-shaped line around the fiber shaft.

The reticulate or hair type of scale structure has the appearance of a rather taunt net stretched across the surface of the fiber. The scales, although slightly curved, appear rather straight and smooth-sided in comparison with those of the wool type. More than two scales meet at a point, and they are often irregular in size and shape.

Hausman [8], from his study of mammalian hair fibers, formulated his well-known classification dividing the scale structure into coronal and imbricate forms. The coronal scales completely encircle the corticle cylinder of the hair shaft, their free ectal edges sculptured in various forms. The imbricate scales do not individually encircle the corticle cylinder, but are arranged like the scales of a fish, with their free distal edges etched into different shapes. The two groups of scales are further classified according to their apparent structure. The coronal scales may be simple, serrate, and dentate; imbricate scales may be ovate, acuminate, elongate, crenate, and flattened. Although the author did not include wool in his studies, the crenate and flattened types are often encountered on wool fibers.

Review of Literature

Although scale structure has not been studied extensively in the field of wool technology, various workers have been interested in the examination of scale structure. Their methods may be classified under three main headings—direct examination of the fiber under the microscope; half-mounting in media of refractive index similar to that of the fibers; and impression or cast methods in quick-drying media.

The direct examination of the fibers under the microscope was unfavourable because of the non-availability of suitable mounting media, and the optical difficulty in focussing wool due to its tridimensional character.

Half-mounting in media of refractive index similar to that of the fibers was used to overcome some of the above-mentioned difficulties. By immersing the fibers halfway in a mounting liquid, the lower part of the fibers becomes invisible because of the liquid: only the fiber surface facing the observer is visible and can be studied. Different media and techniques have been employed by Manby [14], Reumuth and Schwerdiner [18], and Herzog [10], with satisfactory results.

The cast or impression method involves obtaining imprints of the surface structure in a suitable quick-drying media, and then studying the imprints under the microscope or by projection. Various media, such as resin, fingernail polish, cellulose, gelatin, and thermoplastic film, have been suggested by different workers. Saxinger [19], Herzog [9], Von Bergen and Mauersberger [21], Lockte [13], Hardy [4], Hardy and Plitt [5], Koonz and Strandine [11], and Wildman [22] employed different techniques to get cast impressions.

Earlier workers, such as Brevoort [2], McMurtrie [16], Hausman [6], and Manby [14], used special staining techniques for the study of scale structure; these methods have lost popularity due to improvement of the impression method. Only a few references are available regarding the specific scale patterns of different fibers.

Hausman [6], while classifying the scale forms [8] from his drawings of the hairs of 166 fur-bearing animals [7], was able to find an interesting and surprising relationship in the scales of infrahominid mammals—namely, that the size of the scales (*i.e.*, their anteroposterior width) and their forms varied together in a constant way. Moreover, the size (and hence also the forms) of the scales bore a relationship not to the species of mammals bearing the hair but to the diameter of the hair shaft bearing the scales. In the finest and the softest hairs, only the coronal type of scales is found. In fibers less than $8{\cdot}50\mu$ in diameter the scales are coronal, while in fibers coarser than this they are imbricate. Hausman found that there was a decrease in the " scale index "$\left(\frac{\text{scale height}}{\text{diameter}}\right)$in the fibers as the diameter increased. This indicated that there was a pulling-out of the scales with increase in diameter. He also found that cuticle scales may vary according to the cross section of the fiber. In circular and eliptical cross sections, the form is different from that in a flattened cross section, which is like a duck bill where the tip has a flattened portion and is different from the middle and base. The differences in form, according to Hausman, are the results of the differences in the gradual drying-out of the hair shaft plus the effects of wear on the free ectal edges of the scales, particularly at the tips of the shaft.

Litterscheid and Abeler [12] modified the method of accentuating the scales by preliminary treatment of the fibers: by bleaching pigmented hairs, if necessary, with 10 per cent hydrogen peroxide for from 3 to 7 days; or by soaking the fibers in 60 per cent to 63 per cent nitric acid for 6 hrs. and drying. They stained the fibers with carbol fuchsin so that a deposit of dye was left along the edges of the scales on drying. From drawings of this material they delineated and described in detail parts of the hairs of fur-bearing animals.

Butarin [3] made a study of cuticular scales of the fleeces of wild sheep (Argali Arhar) and local fat-rumped sheep in Russia (Kirghiz sheep), and noticed that the cuticular scales of kemp fibers are polyhedral, mostly hexagonal, and form a regular symmetrical pattern. The transitional and downy fibers were found to have annular scales. He stated further that the scales of wild sheep resemble one another but differ from those of domestic sheep.

Skinkle [20], from the measurement of visible scale height and the quantity S^3/D, where S represents the scale height and D the diameter of the fiber, was able to differentiate between wool and mohair fibers as follows:

1. The value of S is below $17{\cdot}0\mu$ in the case of wool fibers.
2. The value of S is above $18{\cdot}5\mu$ in the mohair fibers.
3. If the value of S is between $17{\cdot}0\mu$ and $18{\cdot}0\mu$, the hair is probably wool. This could be verified by determining the value of S^3/D, which is below 140 for wool fibers.
4. If the value of S is between $18{\cdot}0\mu$ and $18{\cdot}5\mu$, the hair is probably mohair. This could be verified by calculating the value of S^3/D, which is above 160 for mohair fibers.

Skinkle stated further that no cases had arisen in his experience which did not fall into one of the above classes, but it is possible that some wools may have values of S slightly above 18μ but values of S^3/D of 140 or less.

Mirzoeva [17] made microscopic investigations of the form and development of the scales of the wool of purebred Merino, Turkmenian fat-rumped, lomud, and Erek sheep, and concluded that the number of scales for a given length of wool (256μ) and the form and area (in μ^2) are specific to the individual breeds studied and must be regarded as genetically controlled. He stated further that, contrary to the findings of D. Udolskii (whose methods were used in his study), no definite influence of breed on the number of rows of scales on a hair could be ascertained.

Mathews [15] stated that the number of scales necessary to cover the circumference of the fiber varies considerably, depending upon the diameter of the fiber. The average height of the scales is approximately 28μ and the average width approximately 36μ. In the finest wools each one of the scales is large enough to encircle the shaft of the fiber, but with increased fiber diameter the number of scales necessary to cover the circumference increases proportionately. Mathews further asserted that the visible scale height in fine wools is from 8μ to 10μ and in coarse wools it may increase to 18μ. The number of scales along the edge of the fiber per 100μ length averages 10 or 12, but may range from 6 to 14.

The origin of the samples used in the present study are given in detail in Appendix Table I and may be summarized as follows:

Fleece type	No. of samples by origin				
	U. S.	England	Asia	Australia	Total
Wild life	4	0	0	0	4
Fine-wool breeds	2	0	0	1	3
Medium-wool breeds	10	2	0	0	12
Long-wool breeds	4	2	0	0	[illegible]
Crossbred breeds	3	0	0	0	[illegible]
Mixed-wool breeds	1	4	28*	0	33
TOTAL	24	8	28	1	61

* India —12 samples
China — 9 samples
Turkey— 4 samples
Austria— 1 sample
Russia — 2 samples

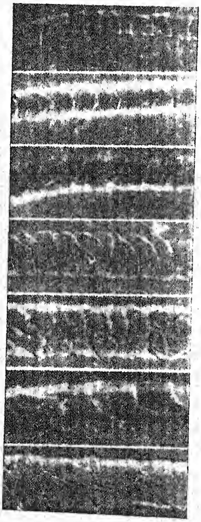

FIG. 1. *Common pattern of scales revealed by surface structure of wool fibers. Coronal, or wool type of scales, and imbricate forms are represented.*

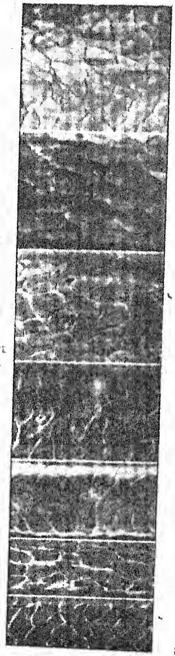

FIG. 2. *Common pattern of scales revealed by surface structure of hair fibers. Polygonal plate and hexagonal and [illegible] forms are represented.*

Preparation of the Samples and Method of Study

Samples from which representative fibers were taken for the study of surface scale structure were first scoured by the emulsion method, using soap and soda, and then rinsed in water. After drying at room temperature, they were given a bath in carbon tetrachloride.

Hardy and Plitt's [5] thermoplastic film technique was followed for determining the surface scale structure on the different types of wool. Impressions were obtained of representative fibers, and photomicrographs were prepared with the help of a projector with a magnification of 500×. The following measurable characters were studied:

1. Form and outline of the scales.
2. Number of scales per 1-in. length of the fiber along one surface.
3. Number of scales per 1-in. length and 100μ width of the fiber, calculated to facilitate a better comparison of different wools on a standard basis.
4. Average scale height in different types of wool.
5. Number of scales per 100μ length along the margin of the fiber.
6. Diameter of the fiber (on the photomicrograph).
7. Relation of scale height to fiber diameter, $\frac{S}{D}$, which Hausman [8] designated as the scale index in his study of fur fibers. The types of scale outline were classified according to that described by Blyth [1] and Hausman [8].

The number of scales per unit length of the fiber were counted (from the photomicrograph) along one surface of the fiber. The number of scales represented the number of complete scales plus half the number of fractional scales.

The measurements of scale height and diameter were made with a millimeter scale on the magnified image. The average scale height was calculated from 10 scales taken at random.

The number of scales per 100μ length along the margin of the fiber, measured with the help of a millimeter scale, represented the number of scale heights in 50 mm. length along the margin.

The observations are recorded in Appendix Tables I—III.

Summary of Results

The results for the different types of wools are summarized on pages 310 and 311.

The scale outline of the wool fibers varied from coronal to imbricate forms (Figure 1), depending upon the thickness of the fibers. On hair fibers the reticulate form of scales was encountered in all cases, with polygonal and flattened types occurring (Figure 2). In the case of kemp fibers the scale outline was mostly of the flattened type, except for those for the wool types of European origin, which were of polygonal form, resembling a honeycomb structure. (See Figures 3-6.)

Results for the Wool Type of Sheep, including the undercoat of Wild Life and the Wool Fibers of mixed Wools

Sheep or wild life type	No. of scales		Average scale height (μ)	No. of scales along margin per 100μ length	Diameter of the fiber (μ)	Scale index $\left(\frac{\text{scale height}}{\text{diameter}}\right)$
	Per 1-in. length	Per 1-in. length and 100μ width				
Fine wools—						
Average	2,283	15,937	13·2	8·3	14·6	0·906
Maximum	2,400	20,000	15·3	10	16·0	1·017
Minimum	2,200	13,750	12·0	6	12·0	0·750
Crossbred—						
Average	2,550	11,107	12·3	8·7	24·0	0·518
Maximum	3,400	17,000	13·8	11	28·0	0·605
Minimum	2,200	7,857	9·7	6	20·0	0·489
Medium wools—						
Average	2,283	9,502	13·8	8	25·8	0·572
Maximum	3,200	13,000	22·0	10	34·0	0·846
Minimum	1,000	5,555	10·1	4	18·0	0·361
Long wools—						
Average	1,650	4,466	24·1	4·5	36·5	0·686
Maximum	2,700	6,750	30·0	5	42·0	1·000
Minimum	1,100	3,333	20·5	4	30·0	0·512
Mixed wools—						
Average	1,969	11,833	14·9	6·8	17·3	0·893
Maximum	2,700	18,333	21·4	9·0	24·0	1·143
Minimum	1,200	6,000	11·2	4	12·0	0·559
Wild life (undercoat)						
Big Horn	2,400	20,000	12·8	9	12·0	1·066
Deer	2,800	28,000	10·1	10	10·0	1·010
Elk	2,700	20,769	11·0	10	13·0	0·916
Antelope	2,400	18,461	14·2	8	13·0	1·092
All wools—						
Average	2,108	11,696	14·85	7·2	20·9	0·814

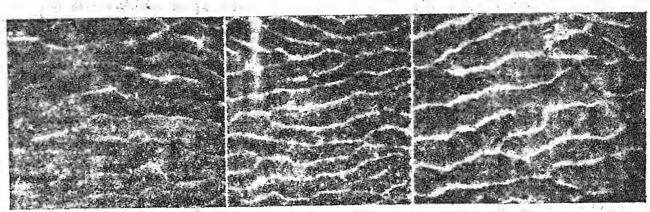

FIG. 3. *Scale type and surface structure of kemp fibres from Asiatic wools.* Left to right : *2 from India and 1 from China. All of flattened type.*

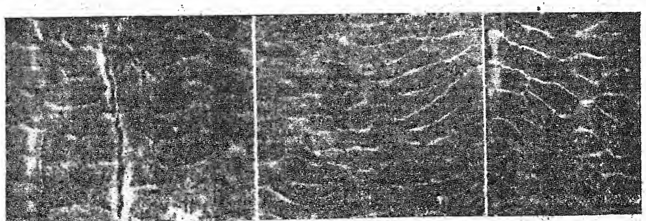

FIG. 4. *Scale type and surface structure of kemp fibres from 3 Asiatic (Turkey) wools. All of flattened type. The fiber on the left was cracked in preparation.*

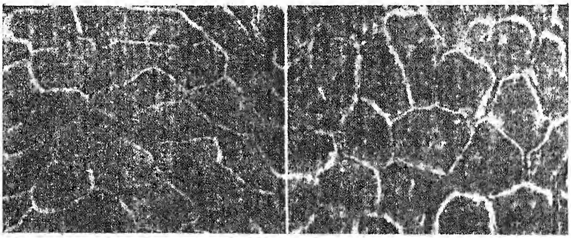

FIG. 5. *Scale type and surface structure of kemp fibres from European wools.* Left: *Herdwick.* Right: *Swaledale. Typical polygonal type resembling a honeycomb is shown.*

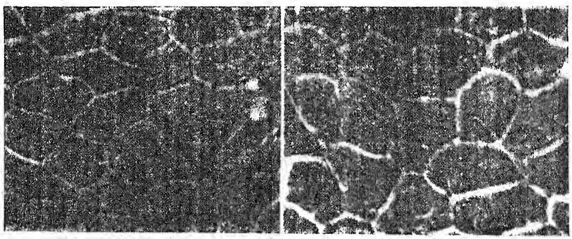

FIG. 6. *Scale type and surface structure of kemp fibres from European wools.* Left: *Scotch Blackface.* Right: *Welsh Mountain. Typical polygonal type resembling a honeycomb is shown.*

Results for Types of Fibers, including the Outer Coat of Mixed-Wool Sheep and Wild Life, such as Kemp Fibers (Hair and Kemp are from Mixed Wools)

Sheep type	No. of scales		Average scale height (μ)	No. of scales along margin per 100μ length	Diameter of the fiber (μ)	Scaleindex $\left(\frac{\text{scale hight}}{\text{diameter}}\right)$
	Per 1-in. length	Per 1-in. length and 100μ width				
Wool—						
Average	2,108	11,696	14·9	7·2	20·9	0·814
Maximum	3,400	20,000	30·0	11	42·0	1·143
Minimum	1,000	3,333	9·7	4	12·0	0·517
Hair—						
Average	3,635	6,001	22·8	5·3	62·0	0·396
Maximum	5,400	10,285	39·5	9	128·0	0·538
Minimum	1,800	3,214	11·5	3	34·0	0·170
Kemp—						
Average	5,383	3,664	18·9	6·8	162·5	0·110
Maximum	7,200	6,500	33·9	10	264·0	0·207
Minimum	3,500	2,500	10·7	4	74·0	0·064
Outer coat of wild life—						
Big Horn	6,000	2,143	24·2	5	280	0·087
Deer	11,500	3,086	25·7	4	376	0·068
Elk	7,600	1,969	24·7	5	386	0·064
Antelope	14,000	2,661	21·2	5·5	526	0·040

Conclusions and Discussion

The following conclusions are evident from the study of surface scale structure in different types of wools:

1. The scale outline in wool fibers of all types can be classified according to the forms of scale structure described by Blyth [1] and Hausman [8]. Serrate and dentate types of scales among the coronal form, and ovate, acuminate, and elongate types of scales among the imbricate form, as described by Hausman [8] on the fur fibers, were not encountered on wool fibers of any type. It was revealed that the scale outline is more closely related to the diameter of the fiber than to the breed or fiber type. Irrespective of the breed or fiber type, the scale type tends to be annular or coronal (single scale across the width of the fiber) up to a diameter of 20μ. As the diameter increases, the form becomes imbricate (more than one scale across the width) (Figure 1). In fibers of greater diameter, such as hair and kemp, the scales show various forms of reticulate and polyhedral structure (Figures 2-6). The scales are fairly regular in the individual fibers but are not specific for breed or fiber type. As the diameter of the fiber increases, the scale height decreases and the form appears to be more flattened than polygonal forms.

2. There is a wide range in the number of scales per 1-in. length of the fiber—the number may vary from 1,000 to 7,000. Irrespective of fiber diameter, the number is smaller in wool fibers (average 2,108) than in hair (average 3,635) and kemp (average 5,383). However, the number of scales per 1-in. length and 100μ width of the fibers is larger in the wool fibers (average 11,696) than in hair (average 6,001) and kemp (average 3,664). Thus, strictly speaking, the number of scales in wool fibers is larger than in hair and kemp. No relationship seems to exist between the number of scales per unit length and the wool type.

3. The average scale height in all types of wools ranged between 9·7μ and 39·5μ (average for wool, 14·85μ; hair, 22·8μ; and kemp, 18·9μ). Contrary to the observations of Skinkle [20] that the average scale height of wool does not exceed 18·0μ, some wool types showed maximum scale heights higher than this limit. Although, on the average, the limit was never exceeded in the fine, cross-bred, or medium-wool types, when taken collectively, the average scale height was higher than the limit in the case of long wools (average 24·1μ). In the case of hair fibers and kemp, the scale height exceeded 18·0μ in many cases. It is therefore doubtful that this criterion for distinguishing between wool and mohair can be relied upon.

4. The number of scales along the margin of the fiber per 100μ length varied from 3 to 11 for all wool and fiber types. The average number of scales on the wool fibers was 7·2; on the hair fibers, 5·3; and on the kemp fibers, 6·8.

5. The scale index [8] was calculated for all wools and fiber types. The range was from 1·143 (maximum for mixed wools) to 0·064 (minimum for kemp). The average scale index for all wool fibers was 0·814; for hair, 0·396; and for kemp, 0·110. There is a distinct difference in scale index between the different kinds of fibers; the scale index for the wool fibers ranged from 0·517 to 1·143; for the hair fibers, from 0·170 to 0·538; and for the kemp fibers, from 0·64 to 0·207. The ranges, as well as the averages, show that the scale index increases progressively in kemp, hair, and wool fibers. Thus, the scale indices differentiate between kemp, hair, and wool fibers but are not accurate for heterotypical fibers. Hausman [8] observed a similar relationship between scale index and diameter of fiber for fur fibers. With an increase in fiber diameter, irrespective of type, there is a corresponding decrease in scale index. The relationship is clearly shown in the Appendix Tables I-III, which include all of the wool and fiber types.

6. There seems to be no relationship of any of the attributes—number of scales per unit length of the fiber, average visible scale height, number of scales per 100μ length along the margin, scale index—to breed or wool type. The observation of Mirzoeva [17] that the number of scales for a given length of wool (265μ) and the form and area (in μ^2) in the purebred Merino, Turkmenian fat-rumped, lomud, and Erek sheep are specific to the individual breeds may be true because of the wide range of variation in the types. However, this generalization is not accurate when the types are more closely related.

7. From the different attributes studied for the scale structure, it appears that the undercoat of the wild species studied was very similar to the undercoat of mixed-wool sheep or the wool fibers of fine-wool breeds, and the outer coat is akin to thin kemp fibers of domestic sheep. The number of scales per 1-in. length

and 100μ width of the fiber was the largest on the undercoat fibers of the deer, numbering 26,000.

8. It is interesting to note that the scale structure of kemp fibers in the four mixed-type wools originating in England (Scotch Blackface, Herdwick, Welsh Mountain, and Swaledale) all showed a similar shape and form—the scales were hexagonal in shape and were combined in a honeycomb-like structure (Figures 5 and 6). On the other hand, kemp fibers from mixed type of wools from Asiatic countries all had the flattened and reticulate type of scales (Figures 3 and 4). It is probable that these special characteristics are related to the origin of domestic sheep in Europe and Asia. A study of the outer coat of Mouflon, Argali, and Urial sheep, and of kemp fibers from domestic sheep from these locations is suggested.

Summary

Scale structure and dimensions and fiber thickness were measured on a number of fibers representing the different fiber types from fleeces of several wild life species, including the Big Horn sheep, and of fibers from the various wool types of domestic and primitive sheep, including the fine-wool, medium-wool, long-wool, and mixed-wool types. The scale index, or relation of scale height to fiber thickness, was studied as a means of identification of fiber types and as a breed characteristic. The relationship between scale structure and dimensions in the various animal textile fibers and the identification of the animals from which the fibers came were studied.

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APPENDIX

TABLE I

Scale Characteristics and Outline on Wool Fibers

Country of origin	Type of wool	Type of fiber	Scale outline	No. of scales per in. length	No. of scales per in. length and 100μ width	Average scale height (μ)	No. of scales along margin per 100μ length	Thickness of the fiber (μ)	Scale index ($\frac{\text{scale height}}{\text{fiber thickness}}$)
Wild life—									
U. S.	Big Horn	Undercoat	Annular	2,400	20,000	12·8	9	12·0	1·066
U. S.	Deer	Undercoat	Annular	2,800	28,000	10·1	10	10·0	1·010
U. S.	Elk	Undercoat	Annular	2,700	20,769	11·0	10	13·0	0·916
U. S.	Antelope	Undercoat	Annular	2,400	18,461	14·2	8	13·0	1·092
Fine-wool breeds-									
Australia	Merino	Wool	Annular	2,250	14,062	15·3	6	16·0	0·956
U. S.	Rambouillet	Fine wool	Annular	2,400	20,000	12·2	9	12·0	1·017
U. S.	Rambouillet	Strong wool	Annular	2,200	13,750	12·0	10	16·0	0·750
Medium-w o o l breeds—									
U. S.	Southdown	Wool	Annular	1,000	5,555	14·6	6	18·0	0·811
U. S.	Hampshire	Fine wool	Imbricate, crenate	2,600	11,818	11·4	10	22·0	0·517
U. S.	Hampshire	Coarse wool	Imbricate, crenate	2,200	7,333	13·2	8	30·0	0·440
U. S.	Shropshire	Fine wool	Annular	2,600	13,000	11·0	9	20·0	0·550
U. S.	Suffolk	Fine wool	Annular	2,400	12,000	11·6	8	20·0	0·580
U. S.	Suffolk	Coarse wool	Annular	1,800	6,000	15·6	7	30·0	0·520
U. S.	Cheviot	Fine wool	Imbricate	2,400	10,909	12·5	8	22·0	0·570
U. S.	Cheviot	Coarse wool	Imbricate	2,400	7,059	18·0	6	34·0	0·529
U. S.	Dorset Horn	Fine wool	Imbricate	2,600	10,000	13·1	9	26·0	0·504
England	Kerryhill	Fine wool	Annular	2,400	12,000	12·7	8	20·0	0·635
England	Kerryhill	Coarse wool	Imbricate	3,200	11,428	10·1	10	28·0	0·361

TABLE I—*contd.*

Scale Characteristics and Outline on Wool Fibers—contd.

Country of origin	Type of wool	Type of fiber	Scale outline	No. of scales per in. length	No. of scales per in. length and 100µ width	Average scale height (µ)	No. of scales along margin per 100µ length	Thickness of the fiber (µ)	Scale index (scale height / fiber thickness)
Long-wool breeds—									
U. S.	Romney	Wool	Imbricate, crenate	2,400	8,571	13·7	6	28·0	0·480
U. S.	Lincoln	Wool	Annular	1,100	3,666	30·0	4	30·0	1·000
U. S.	Leicester	Wool	Imbricate	1,400	3,333	21·6	5	42·0	0·514
U. S.	Cotswold	Wool	Reticulate	2,700	6,750	20·5	5	40·0	0·512
England	Dartmoor	Wool	Imbricate	1,400	4,117	24·3	4	34·0	0·714
England	Wensleydale	Wool	Imbricate	1,800	6,923	22·0	4	26·0	0·846
Crossbred breeds—									
U. S.	Corriedale	Fine wool	Imbricate, crenate	3,400	17,000	9·7	11	20·0	0·485
U. S.	Corriedale	Coarse wool	Imbricate, flattened	2,200	7,857	13·8	8	28·0	0·493
U. S.	Columbia	Wool	Annular	2,200	11,000	12·1	10	20·0	0·605
Mixed-wool breeds—									
India	Bikaner 1	Fine wool	Annular	1,800	11,250	17·2	6·5	16·0	1·075
India	Bikaner 1	Coarse wool	Annular	1,500	12,500	18·2	6	24·0	0·758
India	Bikaner 2	Fine wool	Imbricate, crenate	2,000	12,500	16·8	6	16·0	1·050
India	Bikaner 3	Fine wool	Annular	1,900	10,611	16·8	7	18·0	0·933
India	Bikaner 4	Fine wool	Annular	1,800	9,000	16·0	5·5	20·0	0·800
India	Bikaner 5	Fine wool	Annular	1,500	10,000	18·0	6	18·0	1·000
India	Bikaner 6	Fine wool	Annular	1,900	13,571	13·2	8	14·0	0·943
India	Guzerat	Fine wool	Annular	1,900	10,555	14·2	5	18·0	0·789
India	Deccan	Fine wool	Annular	2,000	16,666	13·3	8	12·0	1·108
India	Bellary	Fine wool	Annular	2,200	15,714	11·2	8	14·0	0·800

TABLE I—*concld.*

Scale Characteristics and Outline on Wool Fibers—concld.

Country of origin	Type of wool	Type of fiber	Scale outline	No. of scales per in. length	No. of scales per in. length and 100μ width	Average scale height (μ)	No. of scales along margin per 100μ length	Thickness of the fiber (μ)	Scale index (scale height / fiber thickness)
India	Nilgiri	Fine wool	Annular	2,400	12,000	12·1	8	20 0	0·605
India	Tibet	Fine wool	Annular	1,800	9,000	15·6	8	20·0	0·780
China	Sinning	Fine wool	Annular	2,200	18,333	11·7	8	12·0	0·975
China	Eastern Kansu	Fine wool	Annular	1,500	12,500	17·1	6	12·0	1·425
China	Sinho	Fine wool	Annular	1,800	12,857	13·5	7·5	14·0	0·964
China	Kokonor	Fine wool	Annular	1,700	10,625	16·2	6	16·0	1·012
China	Kansu	Fine wool	Annular	1,200	6,000	21·4	4	20·0	1·070
China	Ninghsia	Fine wool	Annular	1,700	10,625	15·5	5·5	16·0	0·968
China	Suiyuan	Fine wool	Annular	1,800	13,850	14·8	6	13·0	1·057
China	Yungchang	Fine wool	Annular	2,100	15,000	12·3	7	14·0	0·879
Turkey	Akkaraman	Fine wool	Imbricate, crenate	2,400	10,909	12·4	8	22·0	0·564
Turkey	Kizil Karaman	Fine wool	Annular	2,600	13,000	13·1	8	20·0	0·655
Turkey	Kivircik	Fine wool	Annular	1,600	8,000	15·8	7	20·0	0·790
Turkey	Daglic	Fine wool	Imbricate, crenate	2,700	11,250	14·3	7	24·0	0·596
England	Blackface	Fine wool	Imbricate, crenate	2,000	10,000	13·3	7	20·0	0·665
England	Herdwick	Fine wool	Annular	1,600	8,888	18·5	6	18·0	1·028
England	Welsh Mountain	Fine wool	Imbricate, crenate	2,400	10,909	15·0	6	22·0	0·682
England	Swaledale	Fine wool	Annular	2,200	10,000	12·3	8	22·0	0·559
U. S.	Navajo	Fine wool	Annular	2,100	14,000	12·6	9	15·0	0·840
Austria	Zackel	Fine wool	Annular	1,900	13,571	14·8	7	14·0	1·057
Russia	Voloshian	Fine wool	Annular	2,200	10,714	16·0	7	14·0	1·143
Russia	Donskoi	Fine wool	Annular	2,000	14,285	14·0	7	14·0	1·000

TABLE II

Scale Outline and Characteristics on Hair Fibers (Mixed-Wool) Breeds

Country of origin	Type of wool	Type of fiber	Scale outline	No. of scales per in. length	No. of scales per in. length and 100μ width	Average scale height (μ)	No. of scales along margin per 100μ length	Thickness of the fiber (μ)	Scale index (scale height / fiber thickness)
India	Bikaner 1	Heterotype	Reticulate	2,400	6,000	36·6	5·5	40·0	0·915
India	Bikaner 2	Hair	Imbricate, flattened	3,400	4,473	17·0	7	76·0	0·224
India	Bikaner 3	Heterotype	Reticulate, polyhedral	2,200	6,111	36·4	4	36·0	1·011
India	Bikaner 4	Heterotype	Imbricate, crenate	1,800	8,214	22·0	6	56·0	0·393
India	Bikaner 5	Hair	Reticulate, polyhedral	4,200	5,250	25·6	4	80·0	0·320
India	Bikaner 6	Heterotype	Reticulate, crenate	2,000	5,000	24·4	4·5	40·0	0·610
India	Gujerat	Hair	Imbricate, flattened	3,200	7,272	13·2	7	44·0	0·300
India	Deccan	Hair	Reticulate, polyhedral	4,600	5,750	24·5	6	80·0	0·306
India	Bellari	Hair	Imbricate, crenate	3,400	7,082	13·3	8	48·0	0·277
India	Nilgiri	Hair	Reticulare, polyhedral	2,600	7,647	12·1	6	34·0	0·356
India	Tibet	Hair	Imbricate, flattened	3,700	8,809	11·5	9	42·0	0·274
China	Sinning	Hair	Reticulate, polyhedral	3,500	3,723	39·5	3	94·0	0·420
China	Eastern Kansu	Hair	Reticulate, polyhedral	3,800	5,428	34·1	4	70·0	0·487
China	Sinho	Hair	Reticulate, polyhedral	3,600	5,806	26·0	4·5	62·0	0·419
China	Kokonor	Hair	Reticulate, polyhedral	4,800	3,750	21·8	5	128·0	0·170
China	Kansu	Hair	Reticulate, polyhedral	2,400	4,000	23·0	4·5	60·0	0·383
China	Ninghsia	Heterotype	Imbricate, crenate	1,800	4,500	25·8	4	40·0	0·645
China	Sineyuan	Hair	Reticulate, polyhedral	4,000	4,762	26·4	4	84·0	0·314
China	Yung Chang	Hair	Reticulate, polyhedral	4,000	5,000	21·2	5	50·0	0·424
Turkey	Akkaraman	Hair	Reticulate, polyhedral	4,200	6,562	17·4	6	64·0	0·272
Turkey	Kizil Karaman	Hair	Reticulate, polyhedral	4,200	8,400	14·3	5·5	50·0	0·286
Turkey	Kivircik	Hair	Reticulate, polyhedral	4,600	7,666	14·0	7·5	60·0	0·233
Turkey	Daglic	Hair	Reticulate, polyhedral	5,200	8,125	21·8	5	64·0	0·341
England	Blackface	Hair	Reticulate, polyhedral	5,400	6,750	25·3	5	80·0	0·316
England	Herdwick	Hair	Reticulate, polyhedral	2,600	5,000	22·8	5	52·0	0·438
England	Welsh Mountain	Hair	Reticulate, polyhedral	5,400	6,750	20·1	5	80·0	0·251
England	Swaledale	Hair	Imbricate, flattened	3,200	4,571	12·2	8	70·0	0·174
U. S.	Navajo	Hair	Imbricate, crenate	3,200	7,619	15·1	6	42·0	0·360
Austria	Zackel	Hair	Reticulate, polyhedral	4,100	4,881	34·2	3·5	84·0	0·408
Russia	Voloshian	Hair	Reticulate, polyhedral	4,400	6,666	35·5	2·75	66·0	0·538
Russia	Donskoi	Hair	Reticulate, polyhedral	4,800	10,285	20·1	5	46·0	0·437

TABLE III

Scale Outline and Characteristics on Outer Coat Fibers of Wild Life and Kemp Fibers of Mixed-Wool Types

Country of origin	Type of wool	Type of fiber	Scale outline	No. of scales per in. length	No. of scales per in. length and 100μ width	Average scale height (μ)	No. of scales along margin per 100 μ length	Thickness of the fiber (μ)	Scale index (scale height / fiber thickness)
U. S.	Big Horn	Outer coat	Reticulate, polyhedral	6,000	2,143	27·0	5	280·0	0·096
U. S.	Deer	Outer coat	Reticulate, polyhedral	11,600	3,086	25·3	4	376·0	0·067
U. S.	Elk	Outer coat	Reticulate, polyhedral	7,600	1,969	22·3	5	386·0	0·058
U. S.	Antelope	Outer coat	Reticulate, flattened	14,000	2,661	19·3	5·5	526·0	0·037
India	Gujerat	Kemp	Imbricate, flattened	3,500	2,916	11·7	9	120·0	0·097
India	Daccan	Kemp	Imbricate, flattened	5,200	3,059	14·5	9	170·0	0·085
India	Bellari	Kemp	Imbricate, flattened	4,000	5,405	10·8	10	74·0	0·146
China	Sinning	Kemp	Imbricate, flattened	4,200	2,333	15·9	6	180·0	0·088
Turkey	Akkaraman	Kemp	Imbricate, flattened	6,200	3,100	12·8	6	200·0	0·064
Turkey	Kizil Karaman	Kemp	Imbricate, flattened	4,400	4,888	13·5	8	90·0	0·150
Turkey	Kivircik	Kemp	Imbricate, flattened	6,000	3,529	15·0	7	170·0	0·088
Turkey	Daglic	Kemp	Imbricate, flattened	6,500	6,500	10·7	9	100·0	0·107
England	Blackface	Kemp	Reticulate, honeycomb	7,200	2,727	31·1	4	264·0	0·118
England	Herdwick	Kemp	Reticulate, honeycomb	6,000	2,678	33·9	4	224·0	0·151
England	Welsh Mountain	Kemp	Reticulate, honeycomb	5,600	2,500	28·8	5	224·0	0·129
England	Swaledale	Kemp	Reticulate, honeycomb	5,800	4,328	27·7	5	134·0	0·207

ABSTRACT

Two New Varieties of Australorps. Gerick, A. M. (1950). *World's Poultry Science Journal*, **6**, 128-132

THE two new varieties described are: (1) White, (2) Buf. Australorps, popular as remarkable egg producers, are black in colour. They were developed in Australia by selective breeding from Black Orpington fowls (Meat type) of England. In 1931, two of the Black Australorp hens at the Agricultural College, Cape Province, showed colour changes in the feathers, and following the first annual moult in 1932, became pure white in colour (Mutation).

In the following are given the results of experimental matings and close inbreeding :—

A Black Australorp Male was mated to two White Australorp Hens in the parental generation. Seven chicks were hatched and of these one female was pure white at one year of age. In the following year were made Mother X Son and Brother X Sister matings. A total of 96 chicks were reared. Five of the female chicks changed colour by a depigmentation process between 4 to 5 months of age.

In the second filial generation the white variety carried the recessive white factor giving 3 blacks to 1 white. White males are recommended for breeding purposes.

The presence of a lethal factor associated with white in male chicks, many of which died in the shell, has also been discussed.

The standard description of the White Australorp, with the following exceptions, is the same as for the Black variety. Plumage, beak, legs, nails and skin all white, eyes brown or red. The egg production of the Whites was *at par* with that of the Blacks. As a table bird the White Australorp was found superior to the Black variety. It was also better suited to the hot climate of Africa.

Black Australorps in which a few individuals exhibited red or buff feathers in the neck and back, were mated to White Australorps to weaken the black colour and over a period of ten years, buff progeny were produced. By close inbreeding subsequently, the standard characteristics and economic qualities of the buff variety were improved.—[S.G.I.].

Mortality of chicks as affected by the floor litter. Kennard, D. C. and Chamberlin, V. D. (1950). *Worlds Poultry Science Journal*, **6**, 183-187

THE frequent removal of the floor litter from brooder houses was the customary sanitary procedure in the control of Coccidiosis. The losses of chicks experienced from Coccidiosis and other diseases, when the floor litter was removed and renewed

at frequent intervals, amounted to 19 per cent but the losses were reduced to 7 per cent after the use of (soiled) built-up floor litter. Coccidia-infested birds were present in all the pens irrespective of management of the floor litter. By old built-up floor litter is meant the litter that was used for previous broods of chicks, indoors. It requires two or three broods of chicks before the litter may be considered as sufficiently old and suitable to bring down the mortality percentage in chicks. The continuous use of the floor litter for about a year, is required, before the full beneficial effect upon the livability of chicks can be realised.

In three of the four experiments conducted, the mortality of the chicks from all causes was less on old litter. When the chicks received an incomplete ration, *i.e.*, an all plant diet deficient in riboflavin and the unidentified animal protein factor necessary for the normal growth of chickens raised indoors, the effects of the different kinds of floor litter upon the amount of mortality were pronounced. The authors draw the attention of research workers to the urgent need for fundamental research relating to the chemical and biological processes that take place in the old built-up litter.—[S.G.I.].

A Study of Meat Characteristics of Karakul sheep. HANKINS, O. G., HINER, R. L. AND SIMMONS, V. L. (1951). *J. Anim. Sci.* *10*,**2**, 399-410.

KARAKUL sheep are mainly a fur-producing breed, but those falling short of the fur standards are used for meat and wool production. This study with 57 purebred and high-grade Karakul ewes at the Agricultural Research Centre, Beltsville (U.S.A.), provides a basis for improving the meat producing qualities of the Karakul sheep. Conformation, slaughter, measurement of carcass, its cuts and their eating qualities namely aroma, flavour of fat and lean, juiciness, tenderness and resistance to shearing, and the role of separable fat, lean, total edible meat and bone in the production of marketable meat, are described and their effects discussed.

Results showed that dressing percentage varied directly with fatness and live weight at slaughter, the fattest lot dressing more than 50 per cent., Also, carcasses of fatter ewes graded better. Body length and fatness were not correlated. Among the three most popular cuts, loin and rib cuts increased rapidly with fatness to a farther extent than leg cuts. The deposition of large amount of fat in the tail was a major factor for loss in trimming. Increase in fatness had a decrease in lean and bone, but increase in total edible meat. Fatness helped tenderness much more than aroma, but juiciness was little affected.—[S.V.C.].

The Influence of Nutritional Level on Verminosis in Merino Lambs. LAURENCE, G. B., GROENEWALD, L. W., QUIN, J. I., CLARK, R., ORTLEPP, R. J., AND BOSMAN, S. W. (1951). *J. Vet. Res. (Onderstepoort)*, **25**, 121.

A MASS mixed infestation of Haemonchus Contortus and Oesophagostomum, Columbianum larvae dosed to 7 to 8 month old merino lambs kept on two different planes of nutrition caused a peracute fatal verminosis in all the infested animals. A similar infestation when dosed to 10 to 11 month old lambs under identical conditions caused a chronic verminosis. This finding demonstrates the greater susceptibility of young lambs to worm infestation, regardless of their diet,

and stresses the need of preventing mass infestation in young lambs under all conditions. In another experiment, it was observed that an increase of maize ration by 300 gm. a day caused a marked superiority in worm infested sheep as regards their body weight, appetite for roughage, haemoglobin level, fleece weight and wool fibre thickness. The immediate cause of death in cases of acute verminosis was pulmonary oedema. Phenothiazine was not only found to be superior to either tetrachlorethylene emulsion or copper tartarate and copper arsenate mixture as a vermifuge but also appeared to be a promotor of bileflow.—[P.C.S.].

SECTION OF PARASITOLOGY

Critical Tests of Phenothiazine as an Anthelmintic for Horses. Gibson. T. E. (1950). *Vet. Rec.* **62,** 341-342

THE author has shown that the efficiency of anthelmintics assessed on pre-and post treatment egg count is unreliable since the egg production of worms is inhibited after such treatment. The evaluation of phenothiazine in 30 gm. doses against stongyle worms conducted on the lines suggested in the Hall's Critical Test, was based on counting of worms that are eliminated in a representative faecal sample obtained from animals on the second and third day after treatment taking also into account the worms obtained from the bowel washings after slaughtering the animal. It was observed that the drug at this dosage, though effective against small strongyles, had low efficiency against the immature small strongyles. Its efficiency against large strongyles as reported by previous workers was largely due to higher dosages employed by them.—[D.D.N.].

An inactivated vaccine against Newcastle disease. Doyle, T. M. and Wright, E. C. (1950). *British Vet. Jl.*106, 139-161

AN inactivated tissue vaccine against Newcastle disease has been described. It was prepared by inoculating hen's ten days old fertile eggs with virus into the allantoic cavity and using those embryos which died within seventy-two hours and had extensive haemorrhages of the feather follicles. The chick embryos were finely minced and suspended in crystal violet-ethylene glycol.

Most of the vaccine was prepared from either 'Herts' or Turkey strains of virus and both of these proved satisfactory. Further work is however indicated to see if some other strains of higher immunogenic value can be found.

Immunity was established about seven days after vaccination and persisted for at least twelve months, the longest period so far tested. The vaccine did not evince any untoward reaction, paralysis or check in growth, and could be used safely on chick from six weeks of age. It retained its potency for about five months in cold storage (2 to 4° C.).

The vaccine gave rise to virus neutralising antibodies, but did not induce a positive haemagglutination—inhibition reaction—which is a considerable advantage, as it will not interfere with the use of haemagglutination—inhibition test for the detection of naturally infected fowls. From the casual observations there seemed to be no adverse influence on egg production controlled tests however had not been undertaken.

Comparative tests on eggs and fowls to determine the point of inactivation of the virus in the vaccine, during incubation showed that the egg test was less reliable, the failure of the egg test was probably the result of interference in the allantoic sac between inactivated virus and the propagation of the residual active virus. The greater accuracy of the fowl test depended, no doubt, to some extent on the larger inoculum used.—[R.N.S.].

A Dilution Method for the Milk Ring Test in Bovine Brucellosis Detection. HOLM G. C., EVELETH, D. F., AND RHEALT, P. L. (1950). *Vet. Med.* **XLV**, 400-404

THE authors have described a dilution method for the milk ring test in bovine brucellosis. A total of 1,044 milk samples composite of each animal, in 35 herds, were tested undiluted together with sufficient dilutions to reach a negative end point. A constant amount of antigen, a decreasing amount of milk and an increasing amount of diluent comprising of a negative milk selected for its lack of reaction and high butterfat content, were used. The negative diluent was preserved with formalin and stored under refrigeration without affecting the test. Dilutions of 1 : 1, 1 : 5, 1 : 10, 1 : 50, 1 : 100, 1 : 200, 1 : 300, 1 : 400 and 1 : 800 in 1 ml. volumes were employed. Just after making the dilutions one drop of standard milk ring antigen (supplied by the Bureau of Animal Industry, method of preparation of the antigen not mentioned) was added to each tube and thoroughly mixed. The tubes were then centrifuged at 1,500 r.p.m. for 10 minutes. Readings were made according to the standard of Bruhn and of Roepke. The standard adopted by the former author after incubating the tubes at 37°C. for one hour is as follows :—

Negative : The creamlayer, white ; milk below, bluish violet.

Doubtful : Whole column, uniformly bluish violet.

Weekly positive : Bluish violet coloured cream layer forming a ring, the milk column not visibly decolourised.

Positive : Bluish violet ring, milk column perceptibly decolourised.

Strongly positive : Distinct bluish violet ring, milk column totally decolourised.

The results of the tests carried out on 998 cows, on the basis of blood serum and milk ring reactions, and history, vaccination, previous positive reaction and stage of lactation are tabulated. Out of the 998 cows tested 777, with negative blood reactions did not react in a dilution higher than the 1 : 10 with milk ring test except in one case and only 4 in the group of 111 cow tests with suspicious blood titres reacted higher than the 1 : 10 dilution. A total of 110 cow tests appear in the group of reactors. Of these, animals which had not been vaccinated gave milk ring reactions in dilutions of 1 : 50 or higher. The majority of the calf vaccinates gave milk ring test reactions similar to those observed in the negative and suspect calf vaccinate groups. One-third of the adult vaccinate reactors and those with known long standing infection reacted in dilutions below 1 : 50, thereby indicating that they were becoming ' Cease reactors '.

The agglutination inhibition factor, mastitis, colostrum and secretions from dry cows, which may interfere in the ordinary ring test, do not appear to affect the interpretations of milk ring dilution test.—[M.M.S.].

SECTION OF PARASITOLOGY

A Comparison of the Efficacy of Sulphamezathine (Sulphadimethyl Pyrimidine) and Sulphaquinoxaline in the Control of Experimentally induced Caecal coccidiosis in Chicks. KENDALL, S. B. (1950). *Vet. Res.* **62**, 381-382

SULPHAMEZATHINE and Sulphaquinoxaline were compared at concentrations ranging from 0·025 (in water) to 0·25 per cent (in the food), the period of administration being usually from 48th to 120th hour after infection. Both drugs proved ineffective when administered from 72 to 96 hours after infection at a concentration of 0·025 per cent in water. Both Sulphamezathine and Sulphaquinoxaline gave significant protection against acute coccidiosis, at a concentration of 0·1 to 0·25 per cent in the food. Sulphaquinoxaline at a concentration of 0·05 per cent in drinking water proved markedly more effective than Sulphamezathine in limiting mortality from experimentally induced caecal coccidiosis in young chicks.—[G.A.S.].

The fertility of bovine semen in extenders containing sulfanilamide, penicillin, streptomycin and polymyxin. FOOTE, R. H. AND BRATTON, R. W. (1950). *J. Dairy Sci.*, **33**(8), 544-47

FERTILITY rates of bovine semen extended in 3·6 per cent citrate-yolk, containing no antibacterial agent, sulfanilamide, penicillin, streptomycin, polymyxin or a combination of these antibacterial agents were studied. For the trials, bulls having both low and high fertility rates were employed. The treatment means for the per cent non-returns were not significantly different for the high fertility group of bulls. On the other hand, the addition of penicillin, streptomycin or the combination of all antibacterial agents to the extended semen of the low fertility groups of bulls resulted in an increase in the per cent of non-returns rates which was on an average approximately 10 per cent higher than that got using sulfanilamide, polymyxin or no antibacterial agent. The difference in fertility rates between different extenders on the basis of combined data fell just short of 5 per cent level of statistical significance. The authors conclude that the results appear to warrant the use of penicillin, streptomycin, or a combination of these plus polymyxin and sulfanilamide in extenders for bovine semen for the purpose of increasing to a limited extent, the overall fertility level of bovine semen used for artificial insemination.—[S.S.P.].

Inheritance of Susceptibility to Mastitis. LUSH, JAY, L. (1950). *J. Dairy Sci.*, **33**(2), 121-25

COWS coming from 15 herds in the Canterbury District and 12 herds in the Manawatu District reported on page 60 of the 21st Annual Report of the New-zealand Dairy Board, for the year ending 31st July 1945, were classified as ' susceptible ' or as ' resistant ' according as whether they developed mastitis at any age or reached 8 years of age without showing the injection. The incidence for heritability was sought simply through undertaking tests to find out whether susceptible dams produced on an average a higher percentage of susceptible daughters than the resistant dams in the same herd. For this purpose intra-herd regression of daughters

on dams was carried out and to compensate for the possible effect due to variation in total number of classified dams and the proportion of dams in the 2 groups, weighing of data was carried out. The weight finally selected was nk/n+k, where n and k are number of susceptible and resistant dams respectively.

The average inter-herd regression of daughters on dams using the method was 0·259 for the 15 Canterbury herds and 0·161 for the 12 Manawatu herds. The overall average for the 2 groups was 0·191. The calculated estimate of heritability of individual differences in susceptibility to mastitis comes to 0·38 with a range of 0·06—0·70 for 95 per cent confidence interval. Though the author admits that the data are scanty to draw conclusion within narrow limits. The results obtained, indicate good evidence that heredity plays a moderately large part in cow developing mastitis. Selection against cows which are severely affected, or have severely affected sisters or daughters, is suggested as a genetic method of controlling the incidence of mastitis.—[S.S.P.].

Rearing Chicks on Home Grown Feeding-stuffs. Shaw, R. B. and Nighthall, E. W. *World's Poultry Science Journal* **6**, 3, 201-206

THE results indicate that chicks can be reared on a ration without any protein-rich supplement provided they have access to good grass. A hatch of 192 chicks was divided into three equal groups. Group 'A' was fed on Sussex Ground Oats plus minerals, Group 'B' on Sussex Ground Oats, Bean Meal plus minerals, and control Group 'C' on a more normal rearing ration. Cod Liver Oil was included in all rations for the first ten weeks. All mashes were fed dry with grain fed in the evening. The chicks were allowed on to the grass in covered wire runs at ten days old and were moved daily. The average growth of chickens in Groups A and B were slow in the early stages but improved fairly rapidly as maturity was reached. At eight weeks the average weight per chicken in Group 'C' was 9·85 oz. more then in Group 'B' and 8·26 oz. more than Group 'A' (no protein supplement). At twelve weeks the differences in weight were not much greater and at 24 weeks the average body weight of the pullets in Groups A, B and C were 58·94, 60·77 and 71·70 ozs. respectively. Better early growth would probably be obtained if a more palatable vegetable protein were used. It was observed that growing chickens would not graze well unless they were given sufficient palatable foods to keep them contended.—[S.B.].

Significance of the Differences in Digestibility of Feeds by Cattle and Sheep. Cipollon, M. A., Schneider, B. H., Lucas, H. L., and Pavlech, H. M. (1951). *J. Anim. Sci.* **10**, 337-343

IN the United States, although cattle account for 94 per cent of the total feed consumption by ruminants, as many as 71·5 per cent of the digestion trials with ruminants have been conducted on sheep. Generally the efficiency of digestion of feeds by cattle and sheep has been taken to be essentially the same. But on critical investigation of the published data allowing direct comparison of the digestive efficiency of cattle and sheep fed identical rations, it seems that the digestion coefficients for the two species may not be quite interchangeable.

With this possibility in mind statistical study has been made of published data on a total of 27 feeds including dry roughages, silages and concentrates, involving 1912 trials by 386 authors. The data include proximate composition and the digestion coefficients for organic matter, crude protein, crude fibre, nitrogen-free-extract and ether extract and the contents of the total digestible nutrients in each of the three feed classes. To make the comparisons fair covariance adjustment for proximate composition was conducted. In case of dry roughages significant differences between species of animals exist for the digestibility or organic matter, crude fibre and nitrogen-free-extract and the contents of the total digestible nutrients. In case of silages and concentrates, the differences for the two species were significant only for ether extract. Thus the average differences for dry roughages are in favour of cattle on all nutrients and for silages, although significant only for ether extract, indicate that cattle tend to digest silage better than sheep. The average differences in case of concentrates, however, favour sheep on all the nutrients. Significant species-by-feed interactions were also observed for digestibility of proteins from roughages and that of ether extract from concentrates.

In view of the non-interchangeability of the digestibility data for cattle and sheep and the much larger consumption of feed by cattle, it is suggested that more digestibility work be done on cattle rather than on sheep so that greater accuracy may be attained in the application of the data.—[S.S.N.].

Factors Affecting the Utilization of Food by Diary Cows. 1. The Rate of Passage of Food through the Digestive Tract. Balch, C. C. (1950). *Brit. J. Nutrit.* **4,** 361

THIS work was undertaken to investigate factors that may influence the efficiency of digestive process in cows. Experiments were conducted to study the relative rates of passage of hay, chaff, ground hay and concentrates and to find out the causes and effects of different rates of passage.

The method for measuring the rate of passage of food consisted of feeding a small meal of stained food and counting the stained particles in subsequent voidings of faeces. The principal marked food was hay. Excretion curves were drawn to show the percentage of the total residues voided at any time after feeding.

Excretion curves for hay were characterized by an initial appearance of marker in the faeces varying from 12 to 24 hr. after feeding, a slow excretion of the first 10 per cent of the residues followed by a higher rate of excretion typically resulting in the passage of 80 per cent of the residues within 70-90 hr. Excretion then proceeded more slowly and was completed 7 to 10 days after feeding. These curves also showed that in any one cow hay was excreted at a slower rate than any of the other foods studied.

Ground hay given as a small addition to unground hay was invariably excreted more rapidly than the unground hay. Ground hay in diets in which all the hay was ground was usually excreted over a longer period than hay in a similar diet in which the hay was not ground. These differences were due to changes in the movement of the foods in the reticulorumen. Excretion curves for chaffed hay and ground hay mixed with hay showed that finer particles were excreted more rapidly than larger ones ; in some cows chaffed hay behaved like hay, in others like ground

hay. Cotton-seed husks in concentrates were excreted at a considerably faster rate than hay.

Influence of mangolds on the rate of passage of hay through the reticulorumen was not the same with each cow. The difference appeared to be related to the total intake of dry matter. Indirect evidence suggested that the rate of passage of mangolds from the reticulorumen was more rapid than that of hay. Passage of foods through the omasum, the abomasum and the intestines tended to be rapid when the diet included a high proportion of water.

A decreased rate of passage of food through the reticulorumen was accompanied by a raised digestion of crude fibre and a lowered apparent faecal digestibility coefficeint for crude protein and ether extractable substances.—[A.W.Z.]

Procaine Penicillin and Interrupted Penicillin Treatment in Streptococcus agalactiae Mastitis. Hughes, D. L., Christie, G. T., and Farmer, R. K. (1950) *Vet. Rec.*, **62**(2), 11-14

THE authors describe a set of experiments conducted with a view to determining the penicillin level following the udder infusion and also record the comparative therapeutic trials conducted with procaine and calcium penicillin in an infection of *Streptococcus agalactiae*. As a result of the preliminary trials it was found that, whereas, the calciun penicillin persisted in the udder for sixty hours the procaine penicillin was demonstrated for about twelve hours longer than the calcium penicillin.

In the second series of experiments either salt of penicillin was employed in animals confirmed to be having an infection of *Streptococcus agalactiae* on milk sampling. The dose administered comprised 100,000 units per quarter on two occasions at a 72 hours interval. The interval between the two infusions was planned by referring to the earlier literature which suggested that the interrupted penicillin treatment might allow some of the small organisms, referred to as persisters, to multiply during the intermission and thus rendering them susceptible to treatment when it is recommenced. Along with this the penicillin ointment was applied to the teats and to the milkers hands with a view to obviating the chances of skin infection. Thus, out of twenty one cases treated with procaine penicillin two resisted the treatment (90·5 per cent bacteriological cure) and of 25 cases subjected to a similar treatment with calcium penicillin one failed to respond (96 per cent bacteriological cure).—[N.S.D.]

The Resistance of the Young Calf to Disease. McEwen, A. D. (1950). *Vet. Rec.* **66**, 83-93

NEW born calves during their first 2 weeks of life were subjected to variations in management and feeding and the results were computed in terms of survival and general condition. Calves kept under cold and damp conditions did not show increased susceptibility to disease or death, although they had a setback in health and performance. Pooled colostrum kept at 15°C., thawed and pasteurized was an effective substitute for fresh colostrum. Calves deprived of colostrum showed wide variations in susceptibility. Effects of feeding calf serum, colostrum whey, fresh

REVIEW

DAIRYING IN INDIA, 1951. By James N. Warner *Published by MacMillan & Co. Ltd., Calcutta. Pp. 380. Price Rs. 15*

THIS book, published under the auspices of the Indian Council of Agricultural Research, is the second of a series of manuals relating to animal husbandry subjects. It is divided into 15 chapters. The first 3 chapters deal with dairy farming, management of dairy cattle and physiology of milk secretion. The fourth chapter presents information on the composition of milk, factors affecting the same and the nutritive qualities of milk and milk products. The next 2 chapters deal mostly with the general methods of chemical and bacteriological analysis of milk and milk products. Chapter VII is confined to dairy engineering problems connected with the management and maintenance of a dairy herd and the operations of a dairy or creamery. The next chapter covers the principles underlying such common operations as pasteurisation, separation, homogenisation, and cleaning and sterilisation of dairy equipments. Chapters IX and X present comprehensive information on the methods of manufacture, properties and uses of indigenous dairy products. Chapter XI deals with the principles of production of casein, cheese, ice cream, evaporated and dried milks. The next 3 chapters deal with milk production records, dairy book keeping, marketing of milk and cooperative dairying. The last chapter presents some information on goats and other animals as milk producers.

A few statements deserve some modification : Page 24, 'The calf should be permanently marked soon after birth. This may be done by branding.' Branding is not usually done soon after birth. Page 66, 'Of the amino acid present in casein and albumin, at least three are essential.' Ten amino acids are now known to be essential. Page 69, 'The quantity of vitamin D found in milk depends.......... particularly on the amount of green feed'. It is vitamin A which is markedly influenced by the green feed. Page 90, 'In forewarming milk it is heated to as high a temperature as 230°F and held for 5 to 10 minutes.' At such a high temperature the holding period is usually much less. Page 192, 'Trisodium phospate is a valuable cleaning material. Its corrosive action may be largely overcome by adding to it 5 per cent potassium bichromate'. Dichromate is usually used to prevent corrosion by brine and not in cleansing materials.

This book attempts to cover all aspects of dairying in India and as such, a few pages might have been allotted for a description of some important breeds of cattle and systems of breeding (page 3). More information would be useful on the nutritive value of milk (page 71), its supplementary effect, etc. Some attention could have been paid on the computation of simple dairy rations (p. 26), on the method of determination of Reichert value and detection of adulteration in *ghee*, and on the calculation of total solids in milk by Richmond's formula.

Due to the delay in the publication of this book, the information presented by the author in some places have to a certain extent become out of date, but this defect will no doubt be remedied when a new edition is published.

The material is presented logically and in an easily understandable form. The general get-up of the book is good except for a few misprints. The book is well indexed and should prove of value to all who are interested in dairying, especially students, teachers, field workers and those engaged in dairy business. (K.C.S.)

LITERATURE REVIEW ON FATS AND OILS, 1950

(*Published by the Council of Scientific and Industrial Research, New Delhi 1952. Pp. 28*

THIS pamphlet deals with a review of the literature on oils and fats for the year 1950. It is divided into 2 sections. Section 1 deals with hydrogenated fats under different heads, *viz.*, production and trade, chemistry and technology of hydrogenation, nutritive value, applications, identification and analysis. Section 2 pertains to edible oils and fats and the main subheads are production and trade composition and structure, synthesis, technology and processing; deterioration and spoilage; nutrition and metabolism; and lastly methods of analysis.

This pamphlet is presumably intended for research workers and others interested in the field and as such its usefulness is narrowed down because of the late publication. The printing and the get up of the pamphlet is good except for a few misprints. (K.C.S.).

GENERAL ZOOLOGY

BY TRACY I. STORER (*Published by Mc Graw-Hill, University of California at Davis in the Zoological Sciences, Second Edition, 1951. Pp. V-832, with many figures*

THIS well known text book has been so revised and presented in this second edition that the significant developments in Zoology have been incorporated in a form which can be easily understood by students in Colleges and Universities. Till recently Zoology was studied mainly as a descriptive Science with the basic knowledge of comparative anatomy mostly devoid of consideration of functions and mechanistic conceptions of the process of life and all the text books dealt with the subject matter accordingly, but with this change from morphology to Physiology as the basic theme text books have to be written with the same modern trend of thought; and *General Zoology* by Tracy I. Storer is a good example of this change in Text-book writing.

The book shows in its present revised form a considerable care and contains much useful information on almost all topics of Zoology. It is unique in this respect and is up to date. The whole account is well written and illustrated with the most suitable diagrams. Most of the illustrations are original, clear and add greatly to the usefulness of the book.

Part I on General Animal Biology deals comprehensively with the various subjects, notably Organs and Organ systems, Heredity and Genetics, Animal Ecology, Distribution of animals, Organic Evolution, History of Zoology, Classification and International Rules of Nomenclature. The chapter on Heredity and Genetics explains in the most lucid manner the particulars of the subject in the light of most recent advancements. Addition of such subjects as hormones, genetics of human

milk, stored milk, milk whey, cow serum and effects of injections of calf serum, cow serum and colostrum whey have been tabulated and discussed. Feeding large quantities of *Bact. coli.* had no ill effects. Agglutinins against *Brucella abortus* rose higher after injections of immune serum than after feeding, but the titre in both cases dropped by the 3rd week. Bactericidal properties of blood were similar for animals ranging in age from one day to one month and before or after feeding colostrum. The resistance or immunity enjoyed by the calf, either as a result of ingestion of colostrum or acquired in otherways, is discussed. It is suggested that immunity, in so far as *Bact. coli* infection is concerned, is independent of immune bodies to the organism and this immunity is comparable to that of a disease resistant animal. (T.P.B.).

A Comparision of the Effects of Aureomycin, Penicillin and Streptomycin upon Spermatozoan Livability and Control of Bacteria in Bovine Semen. MYERS, R. M. AND ALMQUIST, J. O. (1951). *J. Anim. Sci.*, 10(2), 322-330

THE paper deal with the effect of aureomycin upon spermatozoan motility and the control of bacterial growth in diluted bovine semen. The results are compared with those obtained with penicillin and streptomycin used singly and in combination. Data on stability of aureomycin in stored semen samples is also presented.

The tests were carried out on 24 ejaculates. In all seven concentrations of the drug namely 12·5, 25, 50, 100, 250, 500 and 1,000 μg. per ml. of diluted semen were tried, in addition to the control, and the samples containing 1,000 units each of streptomycin and penicillin used singly and in combination.

12·5 and 25 μg. concentrations were found to be not toxic to the spermatozoan while the higher concentrations certainly were. With 50 μg. concentration the toxic effect was manifest in the later half of 14 days storage period. Levels above 50 μg. though spermicidal, materially retarded bacterial growth during the storage period studied.

Penicillin and streptomycin used singly or in combination showed lesser spermicidal properties than aureomycin hydrochloride ; the latter however, was equally efficacious in controlling bacterial growth. A concentration of 50 μg. per ml. of aureomycin hydrochloride is recommended for routine use at artificial insemination centres where semen is not normally retained for more than 4 days from the time of collection.

During storage in diluted semen, aureomycin was found to be relatively stabler at 4-5°C. than at room temperatures. (S.S.P.)

Spermatozoan Motility as a Measure of Semen Quality. JOHN F. LASLEY (1951). *J. Anim. Sci.*, 10(1), 211-18

THE paper discusses data for 78 ejaculates from seven registered Hereford bulls. Motility was judged on basis of percentage of motile and progressively motile spermatozoa. Techniques are described for making these determinations.

Four distinct groups of spermatozoa are mentioned in both fresh and four days stored semen. Storage increased the dead, live but non-motile, and weakly motile sperm groups, but diminished the progressively motile sperm group, from 51·6 per cent in average fresh semen to 14·2 per cent in semen stored with egg-yolk. As progressively motile sperm cells were alone believed to take part in fertilization, caution in evaluation and use of semen is advised.

Correlations between different groups and the relationship between sperm motility and conception rate are discussed. Percent motile spermatozoa as determined by haemacytometer count was found to be a good index of motility. (S.V.C.).

The use of Lapinised Rinderpest virus vaccine to replace the annual inoculation of cattle previously protected with inactivated formolised spleen vaccine. BROTHERSTON, J. G. AND PURCHASE, H. S. (1952). *B. Vet. J.* 108, 3, 96-100

THE success of lapinised rinderpest virus vaccine having been acclaimed in China, Hong Kong and East Africa, etc., in protecting highly susceptible cattle, a field experiment was conducted by the authors at a farm in Kenya having all high-grade susceptible cattle.

The state of immunity in cattle inoculated 14½ months back with formaline inactivated rinderpest tissue virus was tested by giving 14 cattle a challenge dose of virulent live virus when only 3 animals out of 14 survived. A batch of 13 cattle immunized with formaline inactivated spleen vaccine 13 months and 19 days before was then given an immunizing dose of lapinised vaccine and challenged with live virus 25 days after immunization when only one out of 13 cattle showed thermal reaction. Lastly 120 susceptible weaners, aged 8-12 months, were given lapinised virus vaccine and challenged 25 days later with virulent live virus when all cattle remained normal—one having died of East Coast Fever.

This proved the potency and superiority of the lapinised vaccine over the formalised one. Lapinised vaccine is cheap—one rabbit producing 500-600 cattle doses many thousands of which can be packed in a small container, its antigenic properties can be more accurately and quickly titrated and the reaction being very mild there is no adverse effect on milk yield or pregnancy. Immunity is conferred 84-100 hrs. after vaccination and this rapid protection makes it useful in combating outbreaks. [B.N.G.]

blood groups, populations, human evolution and the relation of genetics to natural selection have been made.

Part II dealing with the morphology and systematics of the various divisions of animal kingdom gives all that is required for a general study. Details needed for a special study of different groups for which special text books are necessary have been rightly avoided. The synopsis of classification, however, does extend down to orders and even to the principal families in some orders. At the end of each chapter a good list or references is given. Chapter on Mankind *i.e.*, Man's place in nature should, however, have been more comprehensive and should have dealt with the evolution of man from the point of view of the recent discoveries of ancient proto-human finds. (H.R.M.).

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ERRATA

Indian Journal of Veterinary Science and Animal Husbandry, Vol. XXI Part III, September 1951

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139	Head line	Mukhrji	Mukherji
139	22	two	Two
144	Table 111, Col. 111 from bottom, 5	420	422
159	Table XVIII Col. 5	percent	C.V. percent
161	Table XIX Col. 4	Body temperature	Body temperature °F
189	2 from bottom	Mem	Minn
203	Table I from bottom	Bangalore	(Bangalore)
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